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Testing of Toxicology and Emissions Sampling Methodology for Ocean Incineration of Hazardous Wastes

Battelle Ocean Sciences, Duxbury, MA

Prepared for

Environmental Protection Agency
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May 88

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16. ABSTRACT The report addresses the development and testing of a system to expose marine organisms to hazardous waste emissions in order to assess the potential toxicity of incinerator plumes at sea as they contact the marine environment through air-sea exchange and initial mixing. A sampling train was designed and tested at EPA's land-based hazardous waste incinerator, using transformer oil as a waste feed. The incinerator was operated under conditions which would be appropriate for at-sea incinerators. The sampling train (Marine Incineration Biological Assessment Sampler--MIBAS) provides a sea water sample containing a plume emission for the marine organisms testing. Five toxicity-test protocols were refined and/or developed for use in this program: (1) a sea urchin fertilization test; (2) a chronic test using macroalgae Champia parvula; (3) a 7-day chronic test using growth and reproduction of the crustacean Mysidopsis bahia; (4) a 7-day growth and survival test with the fish Menidia beryllina; and (5) a 7-day life cycle test using the archiannelid worm Dinophilus gyrocilatus. The results of applying these tests during a hazardous waste burn are given.

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Incinerators	Tests	Stationary Sources		14B
Marine Biology	Sampling	At-Sea Incineration	08A, 06C	
Sea Water	Bioassay	Hazardous Waste	08J	06A, 06O
Waste Disposal			15E	
Toxicity			06T	

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TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS.. iii

LIST OF TABLES.. vii

LIST OF FIGURES... ix

NON-METRIC TO METRIC CONVERSION FACTORS.................................. x

1.0 GENERAL INTRODUCTION AND RESEARCH OBJECTIVES......................... I-1

1.1 INTRODUCTION... I-1

1.2 RESEARCH OBJECTIVES.. I-4

2.0 MATERIALS AND METHODS.. II-1

2.1 DESCRIPTION OF THE EPA RESEARCH COMBUSTION FACILITY.................. II-1

2.1.1 The Liquid Injection Incinerator System............................ II-1

2.1.2 Pollution Control Equipment.. II-1

2.2 DESCRIPTION OF WASTE FEED.. II-3

2.3 SAMPLING METHODS AND SCHEDULE... II-4

2.3.1 Sampling Locations and Scheule..................................... II-4

2.3.1.1 Sampling at Port 1, the West Port................................ II-4
2.3.1.2 Sampling at Port 2, the East Port................................ II-4
2.3.1.3 Sampling at Port 3, the Stack.................................... II-7

2.3.2 Flue Gas Sampling Activities at the Afterburner.................... II-8

2.3.2.1 MIBAS Sampling... II-8
2.3.2.2 Spiked MIBAS and Spiked MM5 Sampling.............................. II-8
2.3.2.3 Flue Gas Method 5 Sampling at the Stack - Location 3... II-11

2.4 SPIKE SOLUTIONS.. II-13

2.5 ANALYTICAL METHODS... II-13

2.5.1 Sample Descriptions.. II-13

2.5.1.1 Waste Feed Samples... II-13
2.5.1.2 MIBAS Samples.. II-13
2.5.1.3 Modified Method 5 Samples.. II-15

TABLE OF CONTENTS
(Continued)

Page

2.5.2 Sample Preparation and Extraction II-15

2.5.2.1 MIBAS Samples II-15
2.5.2.2 Waste Feed Samples II-16
2.5.2.3 Modified Method 5 Extracts II-18
2.5.2.4 Spiked MIBAS Samples II-19
2.5.2.5 Modified Method 5 Sample II-20

2.5.3 Dioxin and Furan Analysis II-21

2.5.3.1 Quality Control Samples II-22
2.5.3.2 Recovery of Internal Standards II-22
2.5.3.3 Quantification II-25

2.5.4 PCB and Chlorobenzene Analysis II-26

2.6 TOXICITY TEST METHODS II-29

2.6.1 Water Quality Adjustments II-29

2.6.1.1 Recovery of MIBAS Sample II-29
2.6.1.2 Water Quality Adjustment II-31
2.6.1.3 Distribution of Toxicity Tests II-32

2.6.2 Toxicity Tests II-33

2.6.2.1 *Menidia beryllina* - Survival and Growth Test ... II-33
2.6.2.2 *Mysidopsis bahia* - Survival, Growth, and Reproduction Test II-34
2.6.2.3 *Champia parvula* - Reproduction Test II-35
2.6.2.4 *Arbacia punctulata* - Sperm Cell Fertilization Test II-35
2.6.2.5 *Dinophylus gyrociliatus* - Life-Cycle Test II-36

3.0 RESULTS III-1

3.1 CHEMICAL RESULTS III-1

3.1.1 Results of Study Sample Analyses (MIBAS, Waste Feed) III-1

3.1.1.1 Chlorobenzene Results in the Study Samples III-4
3.1.1.2 PCDD Results in Study Samples III-4
3.1.1.3 PCDF Results in Study Samples III-6
3.1.1.4 Metals in the Feedstock Samples III-8

TABLE OF CONTENTS
(Continued)

Page

3.1.2 Quality Control Samples (Spiked Sampling Trains, MM5 Extract Splits) III-10

3.1.2.1 PCB Analyses in Quality Control Samples........ III-10
3.1.2.2 Chlorobenzene Analyses in Quality Control Samples........ III-12
3.1.2.3 PCDD Analyses in Quality Control Samples....... III-15
3.1.2.4 PCDF Analyses in Quality Control Samples....... III-19

3.1.3 Emission Levels........ III-21

3.1.4 Mass Spectral Analysis (GC/MS Scans)........ III-25

3.1.5 Analytical Quality Assurance........ III-32

3.1.5.1 Collection and Analysis of Quality Control Samples........ III-32
3.1.5.2 Preparation and Analysis of Laboratory Blanks.. III-33
3.1.5.3 Determination of Laboratory Recovery Spikes.... III-34

3.2 TOXICITY TEST RESULTS........ III-36

3.2.1 Water Quality Adjustment Results........ III-36

3.2.2 Toxicity Tests........ III-36

3.2.2.1 *Menidia beryllina*........ III-36
3.2.2.2 *Mysidopsis bahia*........ III-39
3.2.2.3 *Champia parvula*........ III-39
3.2.2.4 *Arbacia punctulata*........ III-41
3.2.2.5 *Dinophilus gyrociliatus*........ III-46

4.0 DISCUSSION........ IV-1

4.1 TOXICITY TEST........ IV-1

4.2 EMISSION TEST ANALYSES........ IV-3

4.3 OVERALL DISCUSSION........ IV-4

5.0 REFERENCES........ V-1

APPENDIX A. Standard Operating Procedures (Toxicological and Emissions Sampling Methodology)........ A-i

APPENDIX B. Dioxin/Furan Laboratory Procedures and Mass Spectrometric Identification and Measurement of PCBs and Chlorobenzenes as Isomer Groups........ B-i

APPENDIX C. Mass Spectral Data for MM5 Extracts and Composite MIBAS Sample........ C-i

LIST OF TABLES

Page

Table 1.1. Summary of EPA Ocean Incineration Research Strategy.......... I-2

Table 2.1. PCB Incineration Data for 5-11 March 1986.................... II-5

Table 2.2. Sampling Schedule at the Three Locations..................... II-6

Table 2.3. Summary of Seven-Day MIBAS Runs.............................. II-9

Table 2.4. Summary of Spiked MIBAS/MM5 Runs............................. II-10

Table 2.5. Standard Spike Solution Used in the Land-Based Tests........ II-12

Table 2.6. Samples Recovered During Land-Based Incineration Tests and Subjected to Analysis.................................. II-14

Table 2.7. HRGC/HRMS Operating Parameters.............................. II-23

Table 2.8. Exact Masses Used for the Determination of PCDD and PCDF.... II-24

Table 2.9. GC Operating Condition for PCB and Chlorobenzenes Analysis.. II-27

Table 2.10. Summary of MIBAS Sample Dilution During Water Quality Adjustments.. II-30

Table 3.1. PCB Concentration Found in Study Sample Extracts............ III-2

Table 3.2. Chlorobenzene Concentration Found in Study Sample Extracts.. III-3

Table 3.3. Levels of PCDD in Incineration Study Samples................ III-5

Table 3.4. Levels of PCDF in Incineration Study Samples................ III-7

Table 3.5. Metals Analysis of PCB Feed Stock........................... III-9

Table 3.6. PCB Concentration Found in MIBAS Quality Control Sample Extracts... III-11

Table 3.7. PCB Concentration Found in MM5 Quality Control Sample Extracts... III-13

Table 3.8. Chlorobenzene Concentration Found in MIBAS Quality Control Sample Extracts.................................... III-14

Table 3.9. Chlorobenzenes Found in MM5 Quality Control Sample Extracts... III-16

LIST OF TABLES

(Continued)

Page

Table 3.10. Levels of PCDD in Incineration Quality Control Samples...... III-17

Table 3.11. Spiked MIBAS Train Recoveries.................................... III-18

Table 3.12. Levels of PCDF in Incineration Quality Control Samples...... III-20

Table 3.13. Flue Gas Sampled.. III-22

Table 3.14. PCDD Emission Results (ng/DSM m^3).............................. III-23

Table 3.15. PCDF Emission Results (ng/DSM m^3).............................. III-24

Table 3.16. Destruction Efficiencies for Spiked MIBAS Run 1 (3/8/86).. III-26

Table 3.17. Destruction Efficiencies for Spiked MIBAS Run 2 (3/9/86).. III-27

Table 3.18. Destruction Efficiencies for Spiked MMS Run 1 (3/6/86).. III-28

Table 3.19. Destruction Efficiencies for Spiked MMS Run 2 (3/11/86).. III-29

Table 3-20. Extrapolation of CRF PCB Emission Levels to Vulcanus I Levels.. III-31

Table 3.21 Recoveries of Laboratory Spikes............................ III-35

Table 3.22. Water Quality Adjustment Data for MIBAS Samples Collected During the Period of March 5-11, 1986 at the EPA Combustion Research Facility in Jefferson, Arkansas.......... III-37

Table 3.23. Results of the Seven-Day Exposure of Menidia beryllina to MIBAS Samples.. III-38

Table 3.24. Results of the Seven-Day Exposure of Mysidopsis bahia to MIBAS Samples.. III-40

Table 3.25. Results of the Champia parvula Tests Conducted at ERL-N With MIBAS Samples 1, 3, and 5.................................. III-42

Table 3.26. Results of Sperm Cell Test (Arbacia punctulata) with MIBAS Samples During the Period From March 5-11, 1986....... III-43

Table 3.27. Mean Values for Water Quality Data for Dinophilus gyrociliatus Exposure Media for Composite Samples From Each Treatment on Days 1 and 6.................................. III-47

LIST OF TABLES

(Continued)

Page

Table 3.28. Survival and Reproductive Data for *Dinophilus gyrociliatus* at the Termination of the Seven-Day Test.................... III-48

Table 4.1. Summary of Test Results for Toxicity Tests with MIBAS Samples Conducted at EPA Combustion Research Facility, Jefferson, Arkansas.................................... IV-2

LIST OF FIGURES

Figure 2.1. Diagram of Incinerator System with Sampling Port Locations.. II-2

NONMETRIC TO METRIC CONVERSION FACTORS

From	To	Multiply By
Btu/hr	Joules/hr	1054.35
Btu/CF	Joules/cubic meter	3.72340×10^4
Btu/lb	Joules/gram	2.32444
CF	Cubic meters	0.0283168
CFH	Cubic meter/hr	0.0283168
CFM	L/sec	0.471934
Dram	mL	3.696588
Fahrenheit	Centigrade	1.8 +32
Feet	Meters	0.3048
Gallons	Liters	3.78541
Inches	Centimeters	2.54
lb/hr	Kg/hr	0.453592

1.0 GENERAL INTRODUCTION AND RESEARCH OBJECTIVES

1.1 INTRODUCTION

As part of the Environmental Protection Agency's (EPA) overall strategy developed to evaluate both the potential benefits and risks associated with the incineration of hazardous wastes at sea, an overall environmental research strategy has been formulated (U.S. EPA, 1985). This strategy focuses on the acquisition of scientific information and on the development of new techniques designed to expand our knowledge of 1) the chemical nature of incinerator emissions; 2) the behavior and fate of incinerator emission plumes; 3) the possible extent of exposure of marine organisms to these emissions; and 4) the possible environmental consequences of such an exposure. An integral part of this strategy is the formulation of a firm scientific basis for the assessment of environmental risk through the development of a rational, scientifically defensible methodology for this assessment.

This report addresses Items 1, 2, and 3 of Study Area 1 as depicted in Table 1.1. Study Area 1 in the research strategy concerns the development and testing of a system to introduce marine organisms to hazardous waste incinerator emissions in order to assess the potential toxicity of incinerator plumes at sea as they may come in contact with the marine environment through air-sea exchange and initial mixing in seawater. Since this integrated methodology will ultimately be used during ocean incineration research experiments, or "research burns," the development of such methodology must result in a mobile, rugged system suitable for use aboard incinerator ships.

The development and testing of such a system was designed to occur in two levels of study. During the Level 1 studies, which were completed in 1985 (Boehm et al. 1985) the apparatus for the acquisition of incinerator emissions and the mixing of these emissions with an aqueous seawater medium was developed and validated in the laboratory and field-tested using a pilot scale combustion chamber.

TABLE 1.1 SUMMARY OF EPA OCEAN INCINERATION RESEARCH STRATEGY.

Study Area 1 Methods Development and Range Finding	Study Area 2 Use of New Methods At-Sea (Research Burn)	Study Area 3 Additional Tests and Research to Update Risk Assessment
1. Develop emissions sampling technique for toxicity testing.	1. Baseline and environmental monitoring.	1. Emissions transport testing in lab situation.
2. Develop appropriate toxicity tests.	2. Emissions sampling for chemical analysis.	2. Toxicity testing with other organisms and various methods of exposure and exposure times.
3. Obtain toxicity data from a land-based incinerator.	3. Emissions sampling for toxicity tests.	3. Study Area 2 tests using other vessels in research or trial burns.
4. Determine appropriate emissions sampling procedure for subsequent chemical characterization.	4. Transport model development and verification.	4. Study Area 2 tests using other wastes.
		5. Other tests as required.

An emissions sampling train, designed for direct seawater impingement and emission condensation was developed for use in this study to test organic toxicity of incinerator ship emissions collected by techniques representing ocean exposure. The resultant Marine Incineration Biological Assessment Sampler (MIBAS) was laboratory-tested and validated during runs that included gas-phase spikes of Principal Organic Hazardous constituents (POHCs) found in actual PCB waste incinerator emissions. The final sampling train performance was highly repeatable, with an overall capture of approximately 50 percent for characteristic semi-volatile and non-volatile constituents.

During the Level 1 studies, five toxicity-test protocols were refined and/or developed for use in this program: 1) a sea urchin fertilization test; 2) a chronic test using macroalgae Champia parvula; 3) a seven-day chronic test using growth and reproduction of the crustacean Mysidopsis bahia; 4) a seven-day growth and survival test with the fish Menidia beryllina; and 5) a seven-day life cycle test using the archiannelid worm Dinophilus gyrociliatus. These tests were all applied to simulated hazardous waste incinerator emissions and proved suitable for further use.

Water quality adjustments of the MIBAS samples prior to use of the samples in bioassays proved critical due to the high acidity and altered salinity of MIBAS water samples. Of greatest importance were adjustment protocols developed for pH, ionic strength, and ionic composition adjustments. Standard protocols were developed and were reported in Boehm et al. (1985).

Level 2 of this research was designed to test this integrated emissions sampling/marine bioassay system on emissions from a research burn of PCB-containing wastes conducted at EPA's Combustion Research Facility in Jefferson, Arkansas. This so-called land-based testing of the methodology occurred in March 1986. This report presents the results of the Level 2, land-based testing and, together with the Level 1 results previously reported (Boehm et al., 1985), addresses Items 1, 2 and 3 of Study Area 1 of EPA's Ocean Incineration Research Strategy (Table 1.1).

1.2 RESEARCH OBJECTIVES

The overall objectives of Study Area 1 of the Research Strategy were threefold:

1) The development of a method to obtain samples of incinerator emissions (Level 1).

2) The development of a method to introduce these emissions to an appropriate selection of marine bioassays (Level 1).

3) The laboratory and field testing, validation and refinement of this system (Levels 1 and 2).

The final outcome of Study Area 1 of the "Research Strategy" (Table 1.1) should be a validated, documented, reproducible methodology based on a field-tested system. It is important to note that the Study Area 1 program was designed to develop and document these methods, and was not intended to determine the actual toxicity of incinerator emissions. Toxicity of emissions will be determined in later studies to be conducted during at-sea research burns.

2.0 MATERIALS AND METHODS

The land-based experiment was conducted from March 5 to 11, 1986, at EPA's Combustion Research Facility in Jefferson, Arkansas.

2.1 DESCRIPTION OF THE EPA COMBUSTION RESEARCH FACILITY

The U.S. EPA Combustion Research Facility (CRF) is located at the National Center for Toxicological Research (NCTR) in Jefferson, AR. The research facility is operated for EPA by the Acurex Corporation of Mountain View, CA. The facility has two pilot-plant-sized incinerators; a rotary kiln and a liquid injection unit. The incinerators share the pollution control devices. The test incinerator used for this study was the liquid injection unit.

2.1.1 The Liquid Injection Incinerator System

The liquid injection combustor has two major sections, the combustion section and the pollution control section. The combustion section is made up of three parts, the combustion chamber, the interconnecting breech, and the afterburner. The combustor and the interconnecting breech are housed in a cement-block building, while the afterburner is half inside the building and half outside. However, for this test burn the afterburner was not fired but only provided additional stack length. The two sampling ports on the afterburner are located on the outside of the building. A general diagram of the facility is shown in Figure 2.1. Although the combustor was originally designed to operate at 3 MBtu/hr total heat input, it operates more effectively at 2 MBtu/hr heat input. The combustor's auxiliary fuel is propane. However, when a waste feed, which is rich in heat value, is incinerated, the propane fuel rate is subsequently decreased. The waste feed rate for this study was maintained at 110 lb/hr. The waste feed running time was between five and seven hours for each of the seven test days. Residence time through the combustor and the afterburner is estimated at 8 seconds, when

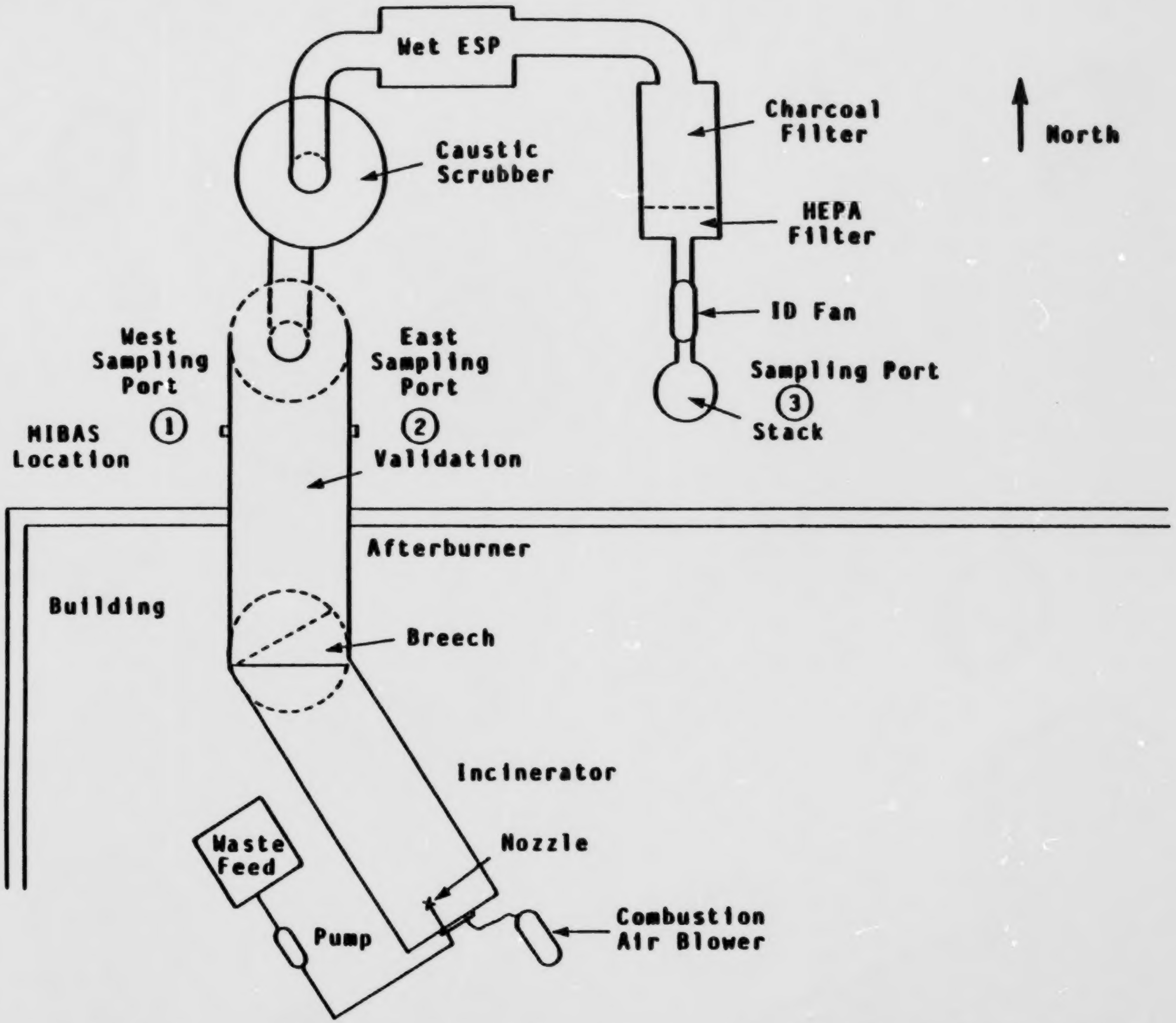
Wet ESP
Caustic Scrubber
Charcoal Filter
HEPA Filter
North
ID Fan
Sampling Port ③
Stack
West Sampling Port ①
East Sampling Port ②
MIBAS Location
Validation
Afterburner
Building
Breech
Incinerator
Nozzle
Waste Feed
Pump
Combustion Air Blower

FIGURE 2.1. DIAGRAM OF INCINERATOR SYSTEM WITH SAMPLING PORT LOCATIONS.

fired at 2 MBtu/hr heat input. The afterburner outlet temperature is typically 2000°F. The inside diameter of each of the combustor's three sections is 34.5 inches. The length of the combustor section is 12.3 feet, the interconnecting vertical breech is 4.8 feet and the afterburner is 14.9 feet for a total of 32 feet.

2.1.2 Pollution Control Equipment

The pollution control section is made up of three separate units: a combined caustic scrubber and temperature quench, a wet electrostatic precipitator (ESP), and an activated charcoal filter. The combustion gases are pulled through the pollution units with an induced draft (ID) fan and into a stack. During the experiment, stack temperatures averaged around 160°F; the stack velocity averaged around 18 feet/s and the volumetric flow rate averaged 625 dry standard cubic feet per minute (DSCF/min). The inside diameter of the stack is 14 inches.

2.2 DESCRIPTION OF WASTE FEED

A source of waste transformer coolant oil containing 50 percent PCBs was obtained from Union Electric Company, St. Louis, Missouri. For these tests, the transformer coolant oil was diluted with an equal amount of No. 2 diesel fuel oil. Two stock batches were prepared in a 250-gallon capacity Tote bulk container during the course of the seven-day test period. The first batch was mixed on March 4 and the second batch on March 9, 1986. After complete mixing, representative samples were collected directly from the bulk container. Samples were always taken while incineration was in progress. Heating values of the feed materials were as follows:

Propane - 2,450 Btu/SCF
PCB/Diesel Mixture (2 Batches):
  5-7 March--13,688 Btu/lb
  8-11 March-14,568 Btu/lb

Incineration data are included in Table 2.1. The chemical content of the waste feed (50:50 transformer oil to No. 2 diesel fuel) is presented in Section 3.1.

2.3 SAMPLING METHODS AND SCHEDULE

2.3.1 Sampling Locations and Schedule

During the seven-day testing period, a series of seven MIBAS samples were collected for use in aquatic toxicity tests. The MIBAS sampler and sampling operation are described in Appendix A to this report. Two spiked MIBAS samples were collected as field validation samples. In addition, EPA Method 5 (M5), ASME/ASTM Modified Method 5 (MM5), and Volatile Organic Sampling Train (VOST) samples were taken.

Table 2.2 summarizes the seven-day sampling activities at each of three sampling locations, the West Port, the East Port, and the stack.

2.3.1.1 Sampling at Port 1, the West Port

At sampling location 1, which is located in the center of the afterburner chamber, two types of sampling systems were used; the MIBAS sampling system and the VOST. The VOST system is described in Hanson, 1984. The MIBAS train is described in Standard Operating Procedures included as Appendix A to this report. The MIBAS sampling, the sample recovery, and the train turn-around were performed exclusively by EPA personnel. The VOST sampling system was operated by Acurex personnel and results are not discussed in this report.

2.3.1.2 Sampling at Port 2, the East Port

At sampling location 2, which is directly opposite Sampling Port 1 and was selected to ensure sampling at equivalent locations, three types of sampling trains were operated on alternate days. The three types of sampling

TABLE 2.1. PCB INCINERATION DATA FOR 5-11 MARCH 1986

Date	Time		PCB/No. 2 Oil Feed Rate Kg/Hr (Lbs/Hr)			Propane Flow Rate CMH (CFH)			Main Chamber Temperature C° (°F)			After Burner Temperature C° (°F)			Stack Temperature C° (°F)		
	Start	Stop	Av[a]	Max	Min	Av	Max	Min	Av	Max	Min	Av	Max	Min	Av	Max	Min
3-5-86	11:04	17:02	48 (105)	62 (136)	36 (80)	3.4 (119)	3.9 (139)	2.6 (92)	1262 (2303)	1301 (2374)	1262 (2016)	982 (1799)	1067 (1953)	912 (1671)	71 (159)	71 (160)	68 (155)
3-6-86	11:06	16:50	50 (110)	67 (148)	27 (60)	2.7 (96)	3 (105)	2.6 (92)	1195 (2183)	1341 (2447)	1088 (1990)	943 (1730)	1060 (1940)	873 (1604)	69 (156)	71 (160)	66 (150)
3-7-86	09:50	14:30	50 (110)	64 (140)	36 (80)	2.7 (96)	3 (105)	2.6 (93)	1239 (2263)	1270 (2317)	1178 (2152)	1012 (1854)	1044 (1912)	973 (1782)	67 (153)	70 (158)	64 (148)
3-8-86	09:45	13.23	45 (100)	53 (111)	40 (88)	2.8 (99)	3 (105)	2.4 (86)	1216 (2220)	1257 (2295)	1181 (2158)	1064 (1948)	1105 (2021)	1045 (1913)	71 (160)	72 (161)	71 (159)
3-9-86	09:15	12:45	52 (115)	58 (128)	47 (104)	2.9 (102)	2.9 (104)	2.8 (100)	1270 (2318)	1363 (2485)	1177 (2150)	1056 (1932)	1106 (2024)	1005 (1841)	71 (160)	72 (162)	70 (158)
3-10-86	10:08	14:45	51 (112)	55 (120)	44 (96)	2.9 (102)	2.9 (104)	2.9 (101)	1267 (2312)	1305 (2384)	1239 (2262)	1064 (1948)	1096 (2004)	1033 (1891)	72 (161)	72 (162)	71 (160)
3-11-86	09:03	13:32	45 (100)	54 (118)	18 (40)	2.9 (101)	2.9 (103)	2.7 (96)	1239 (2263)	1270 (2318)	1208 (2206)	1063 (1945)	1070 (1958)	1043 (1909)	71 (159)	71 (160)	70 (158)

[a] Time averaged.

TABLE 2.2. SAMPLING SCHEDULE AT THE THREE LOCATIONS.

Test Day	Date	Afterburner West Port 1	Afterburner East Port 2	Stack 3
1	Mar 5	MIBAS-01 VOST	MM5	M5, VOST, MM5
2	Mar 6	MIBAS-02	Spiked-MM5	---
3	Mar 7	MIBAS-03 VOST	MM5	M5, VOST, MM5
4	Mar 8	MIBAS-04	Spiked-MIBAS	---
5	Mar 9	MIBAS-05	Spiked-MIBAS	M5
6	Mar 10	MIBAS-06 VOST	MM5	VOST, MM5
7	Mar 11	MIBAS-07	Spiked-MM5	M5

trains are the MM5 trains, the field validation or spiked MIBAS trains, and the spiked MM5 trains. The MM5 train consists of a standard Method 5 sampling train, modified to include a condenser and an XAD-2 resin trap between the filter and the first impinger. The train is operated according to the Method 5 procedures as described in 40 CFR 60.

The field validation (spiked) MIBAS sampling train is essentially a duplicate of the standard MIBAS train, with the exception that a gas-phase injection sub-assembly is placed between the probe and the condenser. A uniformly controlled volume of a spiking solution is injected into the flue gas sample stream. The spiking solution for the validation runs is discussed in Section 2.4.

The "spiked" MM5 sampling train is configured after the MM5, with the exception that a gas-phase injector sub-assembly has been added. The same injector sub-assembly was used for both the MM5 and the field validation (spiked) MIBAS sampling trains.

Acurex personnel operated the MM5 trains on Test Days 1, 3, and 6. Some data from these sampling experiments are included in this report (See Tables 3-16 and 3-17). Battelle personnel operated the spiked MIBAS train on Test Days 4 and 5, and the spiked MM5 train on Test Days 2 and 7.

2.3.1.3 Sampling at Port 3, the Stack

All sampling at location 3 was performed by Acurex personnel. Four, 1-hour M5 tests were run on Test Days 1, 3, 5 and 7. MM5 and VOST samples were taken on Days 1, 3, and 6. These tests were included for permitting purposes. The results are not included in this report.

2.3.2 Flue Gas Sampling Activities at the Afterburner

2.3.2.1 MIBAS Sampling

Table 2.3 summarizes the volume of gas processed by the MIBAS sampling train and the percent moisture in the afterburner gas. The average volume of afterburner flue gas sampled with the MIBAS sampling train was 364 dry standard cubic feet (DSCF) or 10.3 dry standard normal cubic meters ($DSNm^3$). The sampling time for each run was three hours at a nominal sampling rate of 2 actual cubic feet per minute (ACFM). The average moisture content of afterburner flue gas was 9.8 percent. The combustor system ran continuously for the entire seven days with propane as auxiliary fuel. When the sampling of flue gas was in progress, the PCB-containing waste feed was injected at 110 lb/h and the flow of propane was reduced. Sampling usually started only after the combustor had been firing the PCB waste oil for a minimum of 30 minutes. Sufficient propane, about 100 cfh, was fed to the combustor to maintain the temperature at 2300^oF. CO_2 was maintained at 10 percent, and the O_2 at 8 percent. A slight negative pressure was always maintained at the afterburner. The complete combustor operational data are in a report to the EPA (Lee et al., 1987).

The MIBAS impinger water, condensate and the probe rinse were recovered by EPA personnel and were used immediately in the toxicity tests. The unused portions of the MIBAS toxicity test samples were returned to Battelle for chemical analysis. Detailed procedures for the collection of the MIBAS samples are presented in Appendix A.

2.3.2.2 Spiked MIBAS and Spiked MM5 Sampling

Table 2.4 summarizes the sampling data obtained from the field validation (spiked) MIBAS runs and the gas-phase-spiked MM5 runs performed at afterburner Sampling Port 2. These trains were operated by Battelle personnel. The stack gas sample was drawn from a single point inside the

TABLE 2.3. SUMMARY OF SEVEN-DAY MIBAS RUNS

Run Number	MIBAS-01	MIBAS-02	MIBAS-03	MIBAS-04	MIBAS-05	MIBAS-06	MIBAS-07
Date	3/5/86	3/6/86	3/7/86	3/8/86	3/9/86	3/10/86	3/11/86
Clock Time	1133-1433	1129-1445	1003-1303	1004-1304	0932-1232	1019-1319	0920-1220
COMBUSTOR OPERATIONS							
Waste Feed Rate, lbs/hr	105	110	110	100	115	112	100
Propane Feed Rate, cf/hr	119	96	96	99	102	102	101
Temperature, ^{o}C (^{o}F)	1262 (2303)	1195 (2183)	1239 (2262)	1216 (2220)	1270 (2318)	1267 (2312)	1239 (2263)
AFTERBURNER CONDITIONS							
Temperature, ^{o}C (^{o}F)	982 (1799)	943 (1730)	1012 (1854)	1064 (1948)	1056 (1932)	1064 (1948)	1063 (1945)
Gas Flow Rate, DSCF/min	1.97	2.02	2.33	1.94	2.02	1.90	1.98
CO_2-Percent	8.38	6.01	6.20	5.58	7.55	5.49	5.53
O_2-Percent	9.11	12.19	12.64	13.87	10.83	11.88	14.62
MIBAS TRAIN DATA							
Vol. Gas Collected, DSCF	354.35	363.29	418.71	349.52	363.23	341.11	357.08
Vol. Gas Collected, $DSNm^3$	10.03	10.28	11.85	9.89	10.28	9.65	10.11
Moisture, Percent	10.2	8.6	9.7	8.3	11.4	10.9	9.5
Sampling Rate, $DSNm^3$/min	0.057	0.057	0.066	0.055	0.057	0.054	0.056
Sampling Rate, DSCF/min	1.969	2.018	2.326	1.942	2.018	1.895	1.984

TABLE 2.4. SUMMARY OF SPIKED MIBAS/MM5 RUNS

Run No. Date Clock Time	MM5-AS-01 3/6/86 1342-1642	MM5-AS-02 3/11/86 1025-1325	MIBAS-AS-01 3/8/86 1005-1305	MIBAS-AS-02 3/9/86 0932-1232
COMBUSTOR OPERATIONS				
Waste Feed Rate, lb/hr	110	100	100	115
Supplemental Fuel, Propane, cfh	96	101	99	102
AFTERBURNER CONDITIONS				
Temperature, °F	1870	1950	1930	1950
Gas Flow Rate, DSCF/min(a)	253	248	254	244
CO_2, Percent	6.01	5.53	5.58	7.55
O_2, Percent	12.19	14.62	13.87	10.83
"SPIKE" INJECTION OPERATIONS				
Analyte Stock Soln. Conc. µg/100 mL	16	16	16	16
Total Wt. of Analyte Injected, µg	1.54	1.60	1.62	1.60
Vol. of Analyte Per Train, mL	9.6	10.0	10.1	10.0
SPECIFICS FOR EACH TRAIN				
Gas Temperature at Injector	248	268	502	499
Volume of Gas Collected, DSCF	125.6	131.5	313.7	318.6
Volume of Gas Collected, $DSNm^3$	3.55	3.72	8.88	9.02
Particulate Emissions, lb/hr(b)	0.025	0.020	NA	NA
Moisture, Percent	9.0	8.6	7.9	10.7

(a)Based upon an afterburner gas velocity = 3.5 ft/sec., 1990°F, 34.5" dia. and 9.5% moisture.

(b)Based upon filter catch only.

TABLE 2.4. SUMMARY OF SPIKED MIBAS/MM5 RUNS

Run No. Date Clock Time	MM5-AS-01 3/6/86 1342-1642	MM5-AS-02 3/11/86 1025-1325	MIBAS-AS-01 3/8/86 1005-1305	MIBAS-AS-02 3/9/86 0932-1232
COMBUSTOR OPERATIONS				
Waste Feed Rate, lb/hr	110	100	100	115
Supplemental Fuel, Propane, cfh	96	101	99	102
AFTERBURNER CONDITIONS				
Temperature, °F	1870	1950	1930	1950
Gas Flow Rate, DSCF/min(a)	253	248	254	244
CO_2, Percent	6.01	5.53	5.58	7.55
O_2, Percent	12.19	14.62	13.87	10.83
"SPIKE" INJECTION OPERATIONS				
Analyte Stock Soln. Conc. µg/100 mL	16	16	16	16
Total Wt. of Analyte Injected, µg	1.54	1.60	1.62	1.60
Vol. of Analyte Per Train, mL	9.6	10.0	10.1	10.0
SPECIFICS FOR EACH TRAIN				
Gas Temperature at Injector	248	268	502	499
Volume of Gas Collected, DSCF	125.6	131.5	313.7	318.6
Volume of Gas Collected, $DSNm^3$	3.55	3.72	8.88	9.02
Particulate Emissions, lb/hr(b)	0.025	0.020	NA	NA
Moisture, Percent	9.0	8.6	7.9	10.7

(a)Based upon an afterburner gas velocity = 3.5 ft/sec., 1990°F, 34.5" dia. and 9.5% moisture.

(b)Based upon filter catch only.

afterburner, about 10 inches from the inside refractory wall. The combustor operational data for these runs are provided by Acurex in their report to EPA.

Approximately 10 ml of a gravimetrically prepared spiking solution was injected in each of the four runs. The stock spiking solution was identified as BCL No. 41294-2-16 and contained the analytes listed in Table 2.5 at 160 ng/mL (as prepared) in cyclohexane. The target flue gas temperature at the spike injection site was 500°F for the field validation MIBAS runs and 250°F for the spiked MM5 runs. Exact temperatures and spike volumes are given in Table 2.4.

A partial particulate emission rate, based only on the filter catch, is 0.023 lb/h. (This emission rate is not directly comparable to expected emissions at sea, as incinerators used at sea will be larger than the incinerator in use at the research facility.)

For quality assurance purposes, the XAD-2 resin from the MM5 runs, which were collected, extracted, and analyzed by Acurex, was spiked with 200 μL of the internal standard solutions. The standard was prepared at Battelle and contained 2,3,7,8-tetrachlorodibenzo-p-dioxin-$^{13}C_{12}$ (2,3,7,8-tetra-CDD-$^{13}C_{12}$), 2,3,7,8-tetrachlorodibenzofuran-$^{13}C_{12}$ (2,3,7,8-tetra-CDF-$^{13}C_{12}$), and octachlorodibenzo-p-dioxin (octa-CDD-$^{13}C_{12}$) at 1 ng/μL, in n-decane. Each spike was added after the stack sampling and sample recovery were completed. The internal standard was added to the XAD-2 resin from Test Day 1 (Acurex sample No. A03051132A) after an overnight Soxhlet extraction; whereas the remaining two XAD-2 resin samples were spiked directly onto the resin bed before the overnight Soxhlet extraction. The identification number for the Test Day 3 sample is 03071030A, and the number for the Test Day 6 sample is 03071030A. The analysis was completed by the day after sample recovery. An aliquot from each of the three combined MM5 sample extracts was shared between Battelle and Acurex.

2.3.2.3 Flue Gas Method 5 Sampling at the Stack-Location 3

The samples taken for measurements of total particulate emissions by Method 5 were conducted at the stack, location 3, by Acurex. These measurements were taken for permitting purposes. The results will be reported directly to EPA by Acurex and are not discussed further in this report.

TABLE 2.5. STANDARD SPIKE SOLUTION USED IN THE LAND-BASED TESTS

Analyte	Concentration of Spike Solution[a] (ng/ml)	Exact Amount Spiked (μg)			
		MIBAS		MM5	
		Run 1	Run 2	Run 1	Run 2
1,2,4-Trichlorobenzene	160	1.62	1.60	1.54	1.60
1,2,4,5-Tetrachlorobenzene	160	1.62	1.60	1.54	1.60
Pyrene	160	1.62	1.60	1.54	1.60
3,3',4,4'-Tetrachlorobiphenyl	160	1.62	1.60	1.54	1.60
Chrysene	160	1.62	1.60	1.54	1.60
2,2',3,3',5,5',6,6'-Octachlorobiphenyl	160	1.62	1.60	1.54	1.60
Pentachlorophenol	160	1.62	1.60	1.54	1.60
1,2,3,4-Tetrachlorodibenzo-p-dioxin	160	1.62	1.60	1.54	1.60

[a] In cyclohexane.

2.4 SPIKE SOLUTIONS

During this study, both the MIBAS and MM5 train performance was evaluated by the use of gas-phase spiking. The standard spike solution used in both trains was the same concentration and contained the same analytes that had been previously used to validate the MIBAS train in Level 1 studies (Boehm et al., 1985). A summary of these analytes and the concentration of the spike solution is given in Table 2.5.

2.5 ANALYTICAL METHODS

2.5.1 Sample Descriptions

Samples were transferred from the EPA Combustion Research Facility to Battelle's laboratory for processing and analysis. Several sample types recovered from the Arkansas land-based incineration test program were analyzed by Battelle. A summary of the sample types and number of individual samples in each category is given in Table 2.6.

2.5.1.1 Waste Feed Samples

The field samples collected were a 50:50 mixture of Askarel and No. 2 fuel oil. Two fuel samples were collected during the burn tests and analyzed for polychlorinated biphenyls (PCBs), chlorobenzenes, PCDD/PCDF, and metals.

2.5.1.2 MIBAS Samples

The MIBAS samples collected during the field study consisted of seven daily MIBAS train samples used for marine toxicity tests. Three hundred (300) ml of each of these samples were composited, resulting in 2.1 liters of total aqueous material for analysis. This volume represents 7.5 percent of

TABLE 2.6. SAMPLES RECOVERED DURING LAND-BASED INCINERATION TESTS AND SUBJECTED TO CHEMICAL ANALYSIS

Sample Type	Number of Samples	
Feed	2	
MIBAS	1	(Combination of 7 Daily Runs)
MIBAS (Spiked)		
a) Water	2	(Seawater and Seawater Rinses)
b) Rinse	2	(Solvent Rinse)
c) Resin Catch	2	(XAD-2 Resin)
Modified Method 5	3	
Spiked Modified Method 5	2	

the total train samples collected. The remaining sample volumes were used for marine toxicological tests and were archived.

The spiked MIBAS samples represented samples from two trains that were spiked using the gas-phase spiking system to determine recovery of challenge analytes from the authentic flue gas from this study. The spiked MIBAS samples were segregated into three fractions: (1) seawater and seawater rinse (i.e., the "normal" MIBAS sample used for the toxicity tests), (2) wall condensed materials collected as an organic solvent rinse, and (3) the backup XAD-2 resin catch sample. After removing the seawater samples, the train is rinsed consecutively with methanol/methylene chloride, toluene, and methanol to recover any organic materials adhering to the glassware. The backup XAD-2 resin cartridge collects any material that passes through the MIBAS train in the gas-phase.

2.5.1.3 Modified Method 5 Samples

MM5 samples analyzed at Battelle included the extracts from the three MM5 samples collected by Acurex and the two spiked MM5 samples collected by Battelle.

2.5.2 Sample Preparation and Extraction

2.5.2.1 MIBAS Samples

Four MIBAS water samples were extracted for analysis. These samples were

(1) Spiked MIBAS No. 1 (2110 ml volume)
(2) Spiked MIBAS No. 2 (2275 ml volume)
(3) Combined composite of 7 MIBAS runs (300 ml from each for a total of 2100 ml)
(4) Method blank (2000 ml)

The samples were transferred to 4-liter separatory funnels and spiked with 5 ng of each of 2,3,7,8-tetra-CDD/CDF-$^{13}C_{12}$, 1,2,3,7,8-penta-CDD/CDF-$^{13}C_{12}$, 1,2,3,4,7,8-hexa-CDD/CDF-$^{13}C_{12}$, and octa-CDD-$^{13}C_{12}$. The samples were extracted three times with 100 ml portions of methylene chloride.

The combined methylene chloride extracts were concentrated to approximately 25 ml under nitrogen and then transferred to Kuderna-Danish (K-D) concentrator tubes with micro-Snyder columns. The methylene chloride was concentrated to approximately 2 ml, 10 ml of hexane were added and the extract reconcentrated to 2 ml. The four extracts were split in half at this point: one half was used for PCB/chlorobenzenes analysis without further workup and the other half was carried through sample cleanup prior to dioxin/furan analysis.

The subsamples for dioxin/furan analysis were transferred to multilayered silica gel columns. The silica gel columns contained activated silica gel, 44 percent concentrated sulfuric acid on silica gel, with 33 percent 1M sodium hydroxide on silica gel. The columns were rinsed with 90 ml of hexane and the entire volume of eluate was collected. These columns are used to remove acidic and basic compound oxidizable materials from the extracts. The hexane eluates were concentrated to a volume of approximately 2 ml, using a gentle stream of dry nitrogen gas. The hexane solutions were applied to chromatography columns containing approximately 5 g of activated basic alumina. The elution solvents were hexane/methylene chloride (97:3, v/v) and hexane/methylene chloride (1:1, v/v). The 1:1 hexane/methylene chloride eluates were collected, concentrated to near dryness using nitrogen gas, and dissolved in 2 ml of hexane.

The hexane solutions were applied to chromatography columns containing approximately 5 g of activated basic alumina. The elution solvents were hexane/methylene chloride (97:3, v/v) and hexane/methylene chloride (1:1, v/v). The 1:1 hexane/methylene chloride eluates were collected, concentrated to near dryness using nitrogen gas, and dissolved in 2 ml of hexane.

2.5.2.2 Waste Feed Samples

2.5.2.2.1 PCB, PCDD/PCDF Analyses. The two PCB feed samples were prepared for analysis by the following method. The samples were shaken vigorously and 400 µL of each were transferred to 1-dram vials. The weight of each sample was recorded and the samples diluted with 1.60 ml of hexane. The samples were thoroughly mixed with a vortex mixer and 1-ml aliquots removed from each and transferred to other 1-dram vials. One set of sample aliquots was used for PCB/chlorobenzene analysis and the other set was subjected to further sample cleanup prior to dioxin/furan analysis. The sample aliquots used for PCB/chlorobenzene analysis were diluted by a factor of 200:1 prior to analysis without further sample cleanup.

The sample aliquots for dioxin/furan analysis were spiked with 10 ng each of 2,3,7,8-tetra-CDD/CDF-$^{13}C_{12}$, 1,2,3,7,8-penta-CDD/CDF-$^{13}C_{12}$, 1,2,3,4,7,8-hexa-CDD/CDF-$^{13}C_{12}$, and octa-CDD-$^{13}C_{12}$. The sample aliquots were transferred to silica gel columns containing 5 g of 44 percent concentrated sulfuric acid on silica gel. These columns flowed directly into multilayered silica gel columns. The multilayered silica gel columns contained activated silica gel, 44 percent concentrated sulfuric acid on silica gel, and 33 percent 1M sodium hydroxide on silica gel. The columns were rinsed with 90 ml of hexane and the entire eluates were collected.

The hexane eluates were concentrated to a volume of approximately 2 ml, using a gentle stream of dry nitrogen gas. The hexane solutions were applied to chromatography columns containing approximately 5 g of activated basic alumina. The elution solvents were hexane/methylene chloride (97:3, v/v) and hexane/methylene chloride (1:1, v/v). The 1:1 hexane/methylene chloride eluates were collected, concentrated to near dryness using nitrogen gas, and dissolved in 2 ml of hexane.

The hexane solutions were applied to chromatography columns containing approximately 5 g of activated basic alumina. The elution solvents were hexane/methylene chloride (97:3, v/v) and hexane/methylene chloride (1:1, v/v). The 1:1 hexane/methylene chloride eluates were collected, concentrated to near dryness using nitrogen gas, and dissolved in 2 ml of hexane.

The hexane solutions were chromatographed through columns containing approximately 5 g of Florisil. The elution solvents were hexane, 6 percent ethyl ether in hexane and 25 percent hexane in methylene chloride. The hexane/methylene chloride eluates were collected, evaporated to dryness using a gentle stream of dry nitrogen gas, and dissolved in 20 µL of n-decane containing 5 ng of 2,3,7,8-tetra-CDD-$^{37}C_{12}$. The 2,3,7,8-tetra-CDD-$^{37}C_{12}$ was used as an absolute recovery standard. All solutions were stored at 0°C and protected from light until analyzed.

2.5.2.2.2 Metals Analysis of Waste Feed. The two waste feed samples were analyzed by Inductively Coupled Plasma Spectrometry (ICP) to determine metals concentrations. The samples were prepared by placing 5 ml of each of the samples in a platinum crucible and heating the sample on a hot plate until a viscous material remained. The crucibles were transferred to a muffle furnace. After initial heating at 200°C, the temperature was raised slowly to 650°C, where it was held for 4 hours. Upon cooling, the residue was dissolved by heating on a hot plate in 2 ml of 1:1 concentrated nitric acid/water. Upon dissolution, the samples were diluted to 25 ml with water and analyzed.

The samples were analyzed on a JY Model 70P Universal ICP manufactured by Instruments SA, Inc. Twenty-four elements were determined in each of the samples.

Radio Frequency Power -- 1.1 KW
Argon Flow -- 1 12 L/min
Nebulizer Flow -- 0.7 L/min
Viewing Height -- 12 mm
Nitrogen Purge -- 1.2 L/min

The data obtained are presented in Section 3.

2.5.2.3 Modified Method 5 Extracts

The three MM5 sample extracts were transferred to K-D concentrator tubes, approximately 10 ml of hexane added, and the extracts concentrated to 2 ml. The three extracts were split in half at this point: one half was used for PCB/chlorobenzene analysis without further workup and the other half was given further sample cleanup prior to dioxin/furan analysis.

The dioxin/furan analysis sample aliquots were spiked with 10 ng each of 1,2,3,7,8-penta-CDD/CDF-$^{13}C_{12}$ and 1,2,3,4,7,8-hexa-CDD/CDF-$^{13}C_{12}$. The spiked sample extracts for dioxin/furan analysis were transferred to multilayered silica gel columns and processed further as indicated in Section 2.5.2.1.

2.5.2.4 Spiked MIBAS Samples

2.5.2.4.1 Spiked MIBAS Seawater Catch and Seawater Rinse Samples. Spiked MIBAS samples were collected from two independent combustion experiments. The analytical preparation steps and analyses are described in Appendix B.

2.5.2.4.2 Spiked MIBAS Organic Rinse Samples. Two separate rinse samples from each of the two spiked MIBAS sampling trains were processed. The rinse samples consisted of a toluene rinse and a methylene chloride/methanol rinse from each sampler. The methylene chloride/methanol rinse was transferred to a 1-liter separatory funnel and the methylene chloride/methanol layer separated from residual water. The water was extracted three times with 60 ml of methylene chloride and the extracts combined with the methylene chloride/methanol layer. The combined extracts were transferred to a 500 ml round-bottom flask and spiked with 5 ng each of 2,3,7,8-tetra-CDD/CDF-$^{13}C_{12}$, 1,2,3,7,8-penta-CDD/CDF-$^{13}C_{12}$, 1,2,3,4,7,8-hexa-CDD/CDF-$^{13}C_{12}$, and octa-CDD-$^{13}C_{12}$. The toluene rinse was transferred to a separatory funnel and the toluene separated from the residual water. The toluene was added to the combined methylene chloride/methanol rinse and extract. The residual water was extracted three times with 60 ml of methylene chloride and the extracts combined with the methylene chloride/methanol rinse and extract. The volume was concentrated to approximately 10 ml using a Snyder column and then further concentrated to 2.0 ml using a gentle stream of dry nitrogen gas. The two samples were split in half at this point; one half was used for PCB/chlorobenzene analysis without further workup and the other half was subjected to further sample cleanup prior to dioxin/furan analysis.

The sample extracts for dioxin/furan analysis were transferred to multilayered silica gel columns and processed further as indicated in Section 2.5.2.1.

2.5.2.4.3 Spiked MIBAS XAD-2 Resin. The two spiked MIBAS XAD-2 resin samples and a laboratory XAD-2 blank sample were Soxhlet extracted. Because of the large volume of resin in each sample, the extractions were performed in two stages for each sample. Half of the samples were transferred to large Soxhlet thimbles and spiked with 5 ng each of 2,3,7,8-tetra-CDD/CDF-$^{13}C_{12}$, 1,2,3,7,8-penta-CDD/CDF-$^{13}C_{12}$, 1,2,3,4,7,8-hexa-CDD/CDF-$^{13}C_{12}$, and octa-CDD-$^{13}C_{12}$. The samples were extracted with methylene chloride for 18 hours. The extraction thimbles were removed, dried, and emptied, then filled with the remainder of the resin, which was also spiked with 5 ng each of 2,3,7,8-tetra-CDD/CDF-$^{13}C_{12}$ and octa-CDD-$^{13}C_{12}$. The remaining resin was extracted for 18 hours with the methylene chloride from the first extraction. The extracts were concentrated using K-D concentrator tubes and a Snyder column to approximately 2 ml, 10 ml of hexane were added, and the extract concentrated to 2 ml for analysis. The extracts were split in half at this point: one-half was used for PCB/chlorobenzene analysis without further workup and the other half was given further sample cleanup prior to dioxin/furan analysis.

The sample extracts for dioxin/furan analysis were transferred to multilayered silica gel columns and processed further as indicated in Section 2.5.2.1.

2.5.2.5 Spiked Modified Method 5 Samples

The two MM5 sample trains and a laboratory method blank were extracted for analysis. Each MM5 sample train consisted of an XAD trap, particulate filter, and four solvent rinses. The XAD traps were transferred to Soxhlet thimbles and spiked with 5 ng each of 2,3,7,8-tetra-CDD/CDF-$^{13}C_{12}$, 1,2,3,7,8-penta-CDD/CDF-$^{13}C_{12}$, 1,2,3,4,7,8-hexa-CDD/CDF-$^{13}C_{12}$, and octa-CDD-$^{13}C_{12}$. The samples were extracted with methylene chloride for 18 hours. The extract was concentrated to approximately 20 ml using K-D concentrator tubes with a Snyder column. The methanol and methylene chloride rinses were added to the XAD extract and the solution concentrated to approximately 20 ml.

The particulate filters were transferred to Soxhlet thimbles and spiked with 5 ng each of 2,3,7,8-tetra-CDD/CDF-$^{13}C_{12}$, 1,2,3,7,8-penta-CDD/CDF-$^{13}C_{12}$, 1,2,3,4,7,8-hexa-CDD/CDF-$^{13}C_{12}$, and octa-CDD-$^{13}C_{12}$. The filters were extracted with benzene for 18 hours. The extract was concentrated to approximately 20 ml using K-D concentrator tubes with a Snyder column. The toluene rinses were added to the filter extract and the solution concentrated to approximately 2 ml.

The impinger water rinses were transferred to 1-liter separatory funnels and extracted three times with 50 ml of methylene chloride. These extracts were added to the corresponding XAD extracts and the solution concentrated to approximately 2 ml using K-D concentrator tubes with a Snyder column.

The XAD/rinse extracts were combined with their corresponding filter/rinse extracts. One extract was deep green and was diluted to 20 ml with hexane. The other extracts were black and oily and were diluted to 50 ml with hexane to ensure against destruction of the column during cleanup. The extracts were split in half at this point: one set of sample extracts was used for PCB/chlorobenzene analysis without further workup and the other set was given further sample cleanup prior to dioxin/furan analysis.

The sample extracts for dioxin/furan analysis were transferred to multilayered silica gel columns and processed further as indicated in Section 2.5.2.1.

2.5.3 Dioxin and Furan Analysis

The extract splits were analyzed and quantified for PCDD and PCDF. Combined capillary column gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) was used. The HRGC/HRMS consisted of a Carlo Erba Model 4160 capillary column gas chromatograph interfaced directly into the ion source of a VG Model 7070 high-resolution mass spectrometer. The chromatographic column was a 60 M DB-5 fused silica capillary column using helium as the carrier gas. The mass spectrometer was operated in the electron impact ionization mode at a mass resolution of 9000-12000 (M/ M, 10 percent valley definition). The operating parameters of the HRGC/HRMS are summarized in Table 2.7. All HRGC/HRMS data were acquired by multiple-ion-detection (MID), using a VG Model 2035 Data System. The exact ion masses that were monitored are shown in Table 2.8.

2.5.3.1 Quality Control Samples

The operational performance of the HRGC/HRMS was evaluated each day by analyzing standard mixtures of PCDD/PCDF isomers. These standards included mixtures of labeled and unlabeled isomers to evaluate quantification accuracy, mixtures to evaluate congener class chromatographic elution windows, and mixtures of tetra-CDD isomers to evaluate isomer resolution. The mass assignment accuracy was evaluated approximately every four hours by focusing selected ion masses from perfluorokerosine (PFK) and adjusting the slope of the MID unit to correct for minor variations. Mass focus stability was assured by using a reference PFK "lock mass" to correct for drift during the acquisitions.

Method blank samples were processed during the extraction and cleanup of the field samples. These samples demonstrated that contamination was not introduced during the sample workup. The results of these analyses are summarized in Section 3. The method blanks were free of contamination.

2.5.3.2 Recovery of Internal Standards

The recoveries of the internal standards, 2,3,7,8-tetra-CDD-$^{13}C_{12}$, 2,3,7,8-tetra-CDF-$^{13}C_{12}$, and octa-CDD-$^{13}C_{12}$ were calculated by comparison to an external standard, 2,3,7,8-tetra-CDD-$^{37}C_{12}$, which was added following extraction and cleanup. Relative response factors were calculated from

TABLE 2.7. HRGC/HRMS OPERATING PARAMETERS

Mass Resolution	9,000-12,000($M/\Delta M$, 10% Valley Definition)
Electron Energy	70 eV
Accelerating Voltage	6,000 Volts
Source Temperature	200°C
Preamplifier Grain	10^7 Volts/Amp
Electron Multiplier Gain	10^6
Transfer Line Temperature	280°C
Column	DB-5 60m
Injector Temperature	300°C
Column Temperature-Initial	160° Hold for 2 min
Column Temperature-Program	20°/min to 320°C Hold for 20 min
Carrier Gas	Helium
Flow Velocity	30 cm/Sec
Injection Mode	Splitless
Injection Volume	2 µL

TABLE 2.8. EXACT MASSES USED FOR THE DETERMINATION OF PCDD AND PCDF

Compound	Accurate Mass Mass 1	Accurate Mass Mass 2	Theoretical Isotope Ratio Mass 1/Mass 2
Tetra-CDD	319.8965	321.8936	0.77
Tetra-CDF	303.9016	305.8987	0.77
Penta-CDD	355.8546	357.8517	1.54
Penta-CDF	339.8597	341.8567	1.54
Hexa-CDD	389.8156	391.8127	1.23
Hexa-CDF	373.8207	375.8178	1.23
Hepta-CDD	423.7766	425.7737	1.03
Hepta-CDF	407.7817	409.7788	1.03
Octa-CDD	457.7377	459.7347	0.88
Octa-CDF	441.7428	443.7893	0.88
Tetra-CDD-$^{13}C_{12}$	331.9368	333.9338	0.77
Tetra-CDF-$^{13}C_{12}$	315.9418	317.9389	0.77
Penta-CDD-$^{13}C_{12}$	367.8948	369.8918	1.54
Penta-CDF-$^{13}C_{12}$	351.8999	353.8969	1.54
Hexa-CDD-$^{13}C_{12}$	401.8558	403.8529	1.23
Hexa-CDF-$^{13}C_{12}$	385.8609	387.8580	1.23
Octa-CDD-$^{13}C_{12}$	469.7779	471.7749	0.88

triplicate analyses of a standard mixture that contained the four labeled internal standards.

2.5.3.3 Quantification

The PCDD/PCDF isomers were quantified by comparing the sum of the areas of the two ion masses monitored for each congener class to the sum of the areas of the two corresponding internal standard ion masses. The 2,3,7,8-tetra-CDF-$^{13}C_{12}$ was used to quantify the tetra-CDF isomers; the 2,3,7,8-tetra-CDD-$^{13}C_{12}$ was used to quantify the tetra-CDD isomers and the pentachloro and hexachloro PCDD/PCDF isomers. The octa-CDD-$^{13}C_{12}$ was used to quantify the heptachloro and octachloro PCDD/PCDF isomers. Experimental relative response factors were calculated from triplicate analyses of a mixture containing the internal standards and representative isomers from the tetrachlorinated through octachlorinated PCDD/PCDF congener classes. The mixture did not contain a hepta-CDD isomer, so the hepta-CDF response factor was used for quantification of the heptachloro PCDD and PCDF. The response factors were calculated by comparing the sum of the areas for the two internal standard ion masses. The experimental response factors were:

Tetra-CDD	1.16	Tetra-CDF	1.01
Penta-CDD	0.449	Penta-CDF	1.20
Hexa-CDD	0.597	Hexa-CDF	1.02
Hepta-CDD	3.39	Hepta-CDF	3.39
Octa-CDD	1.13	Octa-CDF	1.11

The formula used for quantifying the PCDD/PCDF isomers was

Quantity/Sample =

$$\frac{(\text{Areas of Quantification Masses}) \times (\text{Int. Std. Concentration}) \times 2.5}{(\text{Areas of Int. Std. Masses}) \times (\text{Response Factor})}$$

The following criteria were used to identify PCDD and PCDF isomers:

1) Simultaneous responses at both ion masses.

2) Chlorine isotope ratios within ± 15% of the theoretical value.

3) Chromatographic retention times within windows determined from analyses of standard mixtures.

4) Signal-to-noise ratio equal to or greater than 2.5 to 1.

The 2,3,7,8-tetra-CDD/tetra-CDF and octa-CDD isomers included the additional criterion that they coeluted within 2 seconds of their isotopically labeled analogs. A limit of detection was calculated for samples in which a particular chlorine congener class was not detected. The formula for calculating the detection limit was

$$\text{Limit of Detection/Sample} = \frac{(\text{Heights of Quantification Masses}) \times (\text{Int. Std. Concentration}) \times 2.5}{(\text{Heights of Int. Std. Masses}) \times (\text{Response Factor})}$$

The results from the PCDD/PCDF analyses are summarized in Section 3. A detection limit is listed in parentheses for samples in which a particular compound was not detected. Two samples are reported as containing hepta-CDDs that had chlorine isotope ratios slightly outside the ± 15 percent limit. The retention times and chromatographic characteristics of the peaks obtained in these analyses indicate a high probability that hepta-CDDs were present.

2.5.4 PCB and Chlorobenzene Analysis

All PCB and chlorobenzene analyses were conducted using a Carlo Erba Model 2130 GC coupled to a Finnigan 3200 MS system. This GC/MS system included an INCOS Model 2300 data system that was used to control the MS and to acquire and manipulate data. The instrumental conditions used in this study are given in Table 2.9.

TABLE 2.9. GC OPERATING CONDITION FOR PCB AND CHLOROBENZENE ANALYSIS

Column Coating	SE-54 or DB-5
Film Thickness	0.25 µm
Column Dimensions	30 m x 0.32 mm
Helium Linear Velocity	28-29 cm/sec at 25°C
Initial Temperature	70°C
Initial Time	2 min
Temperature Program	70°C to 310°C at 10°C/min
Chrysene-d_{12} Retention Time	About 21 min
Analysis Time	About 30 min

The sample extract components were separated with capillary column gas chromatography. The PCBs and chlorobenzenes (CB) were identified and measured as isomer groups (i.e., by level of chlorination with electron ionization mass spectrometry). A concentration was measured for each isomer group; total PCB and CB concentration in each sample extract is obtained by summing their individual isomer group concentrations. Nine selected PCB congeners were used as concentration calibration standards. One PCB congener was used as the concentration calibration standard for all isomers at each level of chlorination, except that decachlorobiphenyl was used for all isomers containing nine or ten chlorines. One internal standard, chrysene-d_{12}, was used to calibrate MS response. Six selected CB congeners were used as concentration calibration standards. One CB congener was used as the concentration calibration standard for all of the isomers at each level of chlorination. Phenanthrene-d_{10} was used as the internal standard to calibrate the MS response.

Special Incos software performs automated, unattended identification and measurement of PCBs. Unprocessed GC/MS data are handled without human interaction with the software operating on the dedicated minicomputer. The data obtained from these analyses are presented in Section 3. The data are reported as micrograms found in the total sample except for the PCB feed samples, which are reported in grams per kilograms (g/kg) due to the high concentration of PCBs in these samples. The data acquisition and reduction methods used are described in Appendix B.

The ten largest peaks found in each sample were examined and subjected to a manual computer library search to provide tentative identification of these peaks. The chromatographic, mass spectral, and computer library search data are presented in Appendix C.

2.6 TOXICITY TEST METHODS

2.6.1 Water Quality Adjustments

The aqueous MIBAS samples to be used in toxicity testing were subjected to salinity and pH adjustments. These samples consisted of the contents of the first three impingers, and the probe, flexline and glassware aqueous rinses. The procedures for these tasks are fully described in the SOPs entitled "Recovery of MIBAS Samples Prior to Water Quality Adjustment and Toxicity Testing" and "Water Quality Adjustment of Aqueous Emission Samples"; included in Appendix A of this report.

Because no toxicity was observed during the shakedown tests performed as part of the Level 1 Study (Boehm et al., 1985), all Level 2 studies used a MIBAS mixture at 10 percent strength as the maximum exposure concentration. In contrast, a maximum 2 percent MIBAS concentration was used in all the Level 1 toxicity tests. This change necessitated minor modifications to the SOP entitled "Water Quality Adjustment of Aqueous Emission Samples" in order to prepare enough adjusted MIBAS sample to test at the higher concentration range. A description of the procedures used for the recovery and adjustment of the MIBAS sample follows.

2.6.1.1 Recovery of MIBAS Sample

At the end of each three-hour sampling period during the Level 2 testing, the MIBAS train was disconnected from the probe and delivered to a van that was used for train disassembly, sample recovery, and train cleaning (designated hereafter as the chemistry laboratory). The MIBAS sample consisted of the liquid contents from the first three impingers, the rinse waters from the impingers and glass connectors, and the rinse waters collected from the sampling probe and the flexible tubing which connects the train to the sampling probe (see Table 2.10). The sample was recovered according to the following procedures:

1. A collection bottle was attached to the sampling probe and rinsed three times with North Atlantic Incineration Site (NAIS) seawater ($30^{o}/oo$) while being

TABLE 2.10. SUMMARY OF MIBAS SAMPLE DILUTION DURING WATER QUALITY ADJUSTMENTS.

1. For the worst-case emissions exposure model, the following assumptions are made: (a) a ship with three incinerators is moving at 3 knots (5,556 m/h), (b) the resultant plume has a width of 200 m which falls into the sea directly behind the vessel, and (c) the emissions exit the stack at 66,000 m^3/h and mix to a depth of 50 cm (0.5 m) on contact with the sea.

   - Emissions from 3 incinerators:
     66,000 $m^3/h \times 3 = 198{,}000\ m^3/h$.

   - Volume of seawater contacted by the plume:
     $5{,}556\ m/h \times 200\ m \times 0.5\ m = 555{,}600\ m^3/h$.

   - Therefore,

     $$\frac{198{,}000\ m^3/h\ \text{emissions}}{555{,}600\ m^3/h\ \text{seawater}} = 0.3\ m^3\ \text{gas into}\ 1m^3\ \text{seawater}$$

     $= 0.003\ m^3$ emission gas into 10 L seawater.

   - If a 50% MIBAS collection efficiency is assumed, the worst-case concentration is doubled:

     $0.003\ m^3/10\ L \times 2 = 0.006\ m^3/10\ L$ seawater

     $= 0.0006\ m^3$ emissions per 1 L seawater.

2. The MIBAS sampler collects approximately 10 m^3 emissions over the 3-h sampling period. In the Arkansas test, the 2.3 L MIBAS sample was diluted with seawater to 40 L. If it is assumed that the emissions from the Arkansas incinerator are the same as the at-sea incinerator,

   - 10 m^3 emissions into 40 L seawater
   - = 0.25 m^3 emissions into 1 L seawater. This concentration is 417 times more concentrated than the worst-case estimate.
   - 0.25 m^3/L is referred to as "10% MIBAS."

brushed with a nylon bristle brush. This procedure was repeated for the sampling hose.

2. The probe rinse water was delivered to the chemistry lab and transferred into a 1-gal jug.

3. In the chemistry lab, the sampling train and impingers numbers 1, 2, and 3 were disassembled. The contents of each of the three impingers were transferred to a graduated cylinder and the volume recorded. The impinger contents were then transferred to a prelabeled glass jug. Impingers numbers 1, 2, and 3 were rinsed three times with seawater and the rinse water was added to the sample jug.

4. The rinse water from the sampling probe and the flexline tubing rinses was collected separately and then added to the jug containing the impinger water and rinses.

5. All glass connectors were rinsed with seawater and brushed three times. The condenser was rinsed three times and all rinse water was added to the sample jug. Care was taken to assure that the total sample volume, including the rinse water, did not exceed four liters.

6. The jug containing the sample was capped and delivered to the Mobile Bioassay Laboratory (Mobile Lab) for water quality adjustment. The sample consisted of all the probe and glassware rinse waters, plus the liquids in impingers 1, 2, and 3.

7. The contents of impinger number 4 (NaOH) were transferred to a separate graduated cylinder, the volume was recorded, and the liquid was disposed.

8. Impinger number 5 was weighed with its contents (silica gel) and the weight was recorded.

9. The entire disassembled sample train was solvent-cleaned according to the standard operating procedures included in Appendix A.

2.6.1.2 Water Quality Adjustment

The MIBAS sample, as received at the Mobile Lab (described in detail in Boehm et al., 1985) from the chemistry lab, was transferred to a 4-L graduated cylinder and sufficient seawater (North Atlantic Incineration Site Water diluted to 30°/oo) was added to bring the total volume to four liters. The sample, at this stage, was designated as the "100% unadjusted MIBAS sample." The concentration of emissions in this sample exceeded EPA calculated values for worst-case concentrations of emissions in seawater.

The procedure for water quality adjustment involved salinity and pH adjustments that were necessary to modify the MIBAS sample so that toxic components in the sample could be tested. The salinity and pH of the unadjusted sample (1-liter aliquot) were measured and recorded. The 1-liter aliquot was transferred to a 20-liter carboy and mixed continually by a magnetic stirrer during the water quality adjustment procedure. The salinity was adjusted to approximately 27°/oo with deionized water. Sodium hydroxide was then added to raise the sample pH to 8.0 ± 0.1. If the pH adjustment resulted in a pH >8.1, a small volume of HCl was added to bring the pH back within range. After pH adjustment, the salinity was very close to 30°/oo. Seawater ("NAIS water" at 30°/oo) was then added to bring the sample volume to 10 liters and the sample was thoroughly mixed prior to distribution for toxicity testing. The above procedure resulted in a sample strength of 10 percent MIBAS, which was the highest concentration used in the toxicity tests.

2.6.1.3 Distribution for Toxicity Tests

The 20-liter carboy containing the water quality-adjusted sample was mixed continuously while 250 ml was decanted to a labeled bottle for the sea urchin sperm test and *Dinophilus gyrociliatus* bioassay. The carboy containing 9.75 liters of water quality-adjusted sample was continually mixed while decanting for algal (*Champia*), mysid (*Mysidopsis bahia*), and larval fish (*Menidia beryllina*) toxicity tests. All sample containers and labware were rinsed three times with deionized water and air-dried before being reused.

2.6.2 Toxicity Tests

Standard Operating Procedures for the conduct of all toxicity tests are presented in Appendix A.

2.6.2.1 Menidia beryllina - Survival and Growth Test

The fish test was a seven-day static renewal assay. Six-day-old larvae were exposed to test solutions for seven days. The toxicant tested was the adjusted MIBAS sample. The concentrations tested were 10, 3.2, 1.0, 0.32 and 0.1 percent of the original MIBAS sample. Serial dilutions were made with 30^o/oo NAIS water. There were two controls: sea water collected from the North Atlantic Incineration Site (and diluted to 30^o/oo), and reconstituted Narragansett Bay brine (100^o/oo diluted to 30^o/oo with deionized water). There were three replicates per treatment with 15 fish exposed per replicate. The test chambers were rectangular glass aquaria containing 1000 ml of test solution. Water quality was monitored daily for salinity, temperature and dissolved oxygen, and mortality was recorded. Daily renewals were performed with dilutions of freshly obtained MIBAS samples, or the appropriate control waters. Fish were fed Artemia sp. nauplii daily. At the end of the seven-day period all surviving fish were placed by replicate in 4 percent buffered formalin. Upon returning to EPA Environmental Research Laboratory in Narragansett, RI (ERL-N), they were rinsed with deionized water, dried and weighed. The endpoints for this test were survival and growth (dry weight). ANOVA and Duncan's Multiple Range test were used to determine significance at the 0.05 alpha level.

2.6.2.2 Mysidopsis bahia - Survival, Growth and Reproduction Test

The crustacean test was nearly identical to the fish test. Seven-day-old individuals were exposed for seven days under static renewal conditions. Due to insufficient numbers of organisms, the reconstituted brine control and 0.32 percent concentration were deleted. There were eight replicates per treatment with five individuals per replicate. The exposure chambers were 200 ml plastic cups (pre-soaked in seawater) containing 150 ml of the test solution. Unlike the fish test, upon termination, the surviving animals were placed in uncontaminated NAIS water and brought back to ERL-N alive. There they were examined for the presence of eggs in the oviducts and brood sacs, then dried and weighed. The endpoints were survival, growth (dry weight) and reproductive potential (as percentage of females with eggs in the brood sac). ANOVA and Duncan's Multiple Range test were used to determine significance at the 0.05 alpha level (egg development data was first arc sine root transformed).

2.6.2.3 Champia parvula - Reproduction Test

Over the seven-day period, three separate Champia tests were performed. Each test consisted of a 2-day exposure period (during which test solutions were not renewed) followed by a five- to seven-day recovery period. The concentrations used were identical to those used in the fish test, except that the reconstituted brine control was replaced with a Narragansett Bay seawater control. There were three replicates per treatment. The exposure chambers were plastic cups containing 80 ml of the appropriate test solution plus nutrients and sodium bicarbonate.

To initiate a test, five uniform tips cut from female plants and one larger male tip were placed in each cup. The cups were covered, constantly illuminated and gently swirled four times per day. Only temperature was recorded. At the end of the two-day exposure period, the condition of all tips was noted and the males were discarded. The female tips were then placed in glass recovery bottles (BOD bottles), aerated and constantly illuminated. At the end of the recovery period they were examined for the presence and number of cystocarps (as evidence of sexual reproduction). The endpoints observed were general health and evidence of sexual reproduction (number of cystocarps per individual).

2.6.2.4 Arbacia punctulata - Sperm Cell Fertilization Test

The sperm cell test, using Arbacia punctulata, was conducted with each separate MIBAS sample generated during the period from March 5-11, 1986. One MIBAS sample was generated daily and a minimum of four replicate tests (four different sperm samples) were conducted with each sample. The test procedures followed the standard operating procedure entitled "Conducting Sea Urchin Sperm Cell Toxicity Test" which is included in Appendix A. This test, which can be performed within a few hours, is used to determine the concentration of a test substance that reduces the fertilization of exposed gametes relative to that of control gametes.

Initially, the MIBAS sample was tested at a maximum concentration of 10 percent with 50 percent serial dilutions. This concentration range was found to be too toxic and subsequent tests were run at a maximum concentration of 1 percent MIBAS. Reconstituted brine (diluted to 30°/oo with deionized water) was used as a reference control and NAIS (diluted to 30°/oo) was used as the test control and as diluent for the MIBAS treatments.

Percent fertilization for the control tests for each sample were determined by calculating percent fertilization for individual replicate tests. The mean and standard deviation for percent fertilization were calculated for those replicate tests considered acceptable. EC50 data were obtained by pooling the total egg counts and pooling the unfertilized egg counts for each exposure concentration (excluding rejected replicate test data). EC50 values were calculated according to the procedures outlined in Battelle SOP 7-006-02 entitled "Calculation of LC50 and EC50 Values in Toxicological Studies."

2.6.2.5 *Dinophilus gyrociliatus* - Life-Cycle Test

A seven-day life-cycle test with the archiannelid *Dinophilus gyrociliatus* was also performed. A minimum of 20 one- to two-day-old juveniles were exposed per treatment in Stender dishes with ground glass lids in 10 ml of exposure medium (five replicates per treatment). Eighty percent of the test medium in each test chamber was renewed daily with a fresh MIBAS sample after the biological observations had been made. This test was conducted at 25°/oo salinity with NAIS diluted to 25°/oo used as the control and dilution water. The ten percent MIBAS at 30°/oo salinity was diluted to 25°/oo with deionized water for the highest treatment concentration. The treatments tested in this study were 8.3, 4.2, 2.1, 1.0, 0.5, and 0 percent MIBAS.

Due to the limited volume of test media used in this test, water quality measurements of dissolved oxygen, temperature, salinity, and pH were made on fresh and 24-hour-old composite samples for each treatment on Days 1 and 6. The test chambers were kept in a dark compartment where the temperature was monitored continuously with a temperature probe, except during the observation and sample renewal operations.

The daily observations included counts of number of initial animals surviving, number of eggs laid, number of eggs per egg capsule, number of juveniles released, and, at the termination of the test, the number of eggs visible within the coelom of each worm. The four- and seven-day LC50s were calculated using the moving average method. Reproductive success (as determined by the total number of eggs laid and visible in the coelom of each worm divided by the number of surviving worms for each replicate at the termination of the test) was analyzed by the Student-Newman-Keuls multiple range test for significant differences as compared with the control.

3.0 RESULTS

3.1 CHEMICAL RESULTS

The results of all chemical analyses are presented in this section. The study samples are described first. Bioassay measurements were taken from the MIBAS study sample. The MIBAS sample was composited over seven daily runs and a composite analysis of organic constituents performed. These analysis included polychlorinated biphenyls (PCB), polychlorinated dibenzofurans (PCDF) and polychlorinated dibenzo-p-dioxins (PCDD).

In addition, GC/MS analyses were performed on several of the sample extracts and a mass spectral library search was performed on the MIBAS composite sample to determine if any unusual or known toxic compounds were found in the MIBAS sample used for bioassay. These results, along with analysis of metal levels in the PCB feed mixture, are presented in this section. The results of extensive quality control (QC) analyses are given in a separate section describing these results. Those QC samples include spiked MIBAS, spiked MM5, and MM5 samples.

3.1.1 Results of Study Sample Analyses (MIBAS, Waste Feed)

The results of PCB analyses are given in Table 3.1, and the results of chlorobenzene analyses are given in Table 3.2. For values below the limit of detection, the minimum detection limit for the listed analyte is presented in parenthesis. PCB and chlorobenzene detection limits represent a signal of three times the estimated average noise range. For PCDD and PCDF, this value represents 2.5 times the estimated noise level.

The PCB data shown in Table 3.1 measure total congener class analyses for mono- through decachlorobiphenyl in the feedstock burned, and the MIBAS composite water sample. The feedstock was found to contain 24.5 percent by weight total PCB, principally from the hexa- and heptachlorobiphenyl congener groups. The PCB liquid for this study was a utility askarel, a mixture of approximately 50 percent PCB and 25 percent tri- and tetrachlorobenzene. No detectable PCBs were found in the MIBAS composite sample.

TABLE 3.1. PCB CONCENTRATION FOUND IN STUDY SAMPLE EXTRACTS (NUMBERS IN PARENTHESES INDICATE LEVEL OF DETECTION IN µg FOR THE TOTAL SAMPLE)

Isomer Group	PCB Feed No. 1 (g/kg)	PCB Feed No. 2 (g/kg)	MIBAS Composite Water[a] (total µg)
Monochlorobiphenyl	(0.2)	(0.2)	(0.4)
Dichlorobiphenyl	(0.2)	(0.2)	(0.4)
Trichlorobiphenyl	(0.2)	(0.2)	(0.4)
Tetrachlorobiphenyl	1.0	1.1	(0.8)
Pentachlorobiphenyl	35.0	34.8	(0.8)
Hexachlorobiphenyl	119	116	(0.8)
Heptachlorobiphenyl	73.5	71.4	(1.1)
Octachlorobiphenyl	18.1	16.9	(1.1)
Nonachlorobiphenyl	0.9	1.1	(1.9)
Decachlorobiphenyl	(0.8)	(0.8)	(1.9)
TOTAL	248	241	ND

ND = Not detected.

[a] Sample volume was 2.1 Liters.

TABLE 3.2. CHLOROBENZENE CONCENTRATION FOUND IN STUDY SAMPLE EXTRACTS (NUMBERS IN PARENTHESES INDICATE LEVEL OF DETECTION IN µg FOR THE TOTAL SAMPLE)

Isomer Group	PCB Feed No. 1 (g/kg)	PCB Feed No. 2 (g/kg)	MIBAS Composite Water[a] (µg/mL)
Chlorobenzene	(0.2)	(0.2)	(0.4)
Dichlorobenzene	4.8	4.6	(0.4)
Trichlorobenzene	116	124	(0.6)
Tetrachlorobenzene	12.7	12.7	0.5
Pentachlorobenzene	(0.4)	(0.4)	1.3
Hexachlorobenzene	(0.5)	(0.5)	1.3
TOTAL	134	141[a]	3.1

[a] Sample volume was 2.1 liters.

3.1.1.1 Chlorobenzene Results in the Study Samples

The chlorobenzene results in the study samples, shown in Table 3.2, are very interesting. The total chlorobenzene levels in the feedstock, 13.4 percent (w/w) and 14.1 percent (w/w), are consistent with an askarel containing a 25 percent (w/w) chlorobenzene mixture, that is diluted by half with fuel oil. This dilution with fuel oil occurred in the feedstock for the land-based burns. These data reveal an appreciable level of dichlorobenzene in the liquid burned. The dichlorobenzene was not anticipated.

The MIBAS composite sample was found to have measurable levels of tetrachlorobenzene, pentachlorobenzene, and hexachlorobenzene in the seawater catch that was used for bioassay testing. This result is interesting because, of the di, tri- and tetrachlorobenzenes, the predominant materials in the combustion feedstock, only the tetrachlorobenzene was observed at a very low level in the MIBAS samples (0.5 µg/L). This result may indicate that higher chlorinated materials are more efficiently collected by the MIBAS train.

The relatively higher levels of pentachlorobenzene and hexachlorobenzene in the MIBAS sample may also indicate that decomposition of PCB to these products, or chlorination of the tri- and tetrachlorobenzenes to higher chlorinated analogs, may be occurring in the flame processes. Since these higher chlorinated benzenes with five or more chlorine atom substituents are not found in the fuel, they must be either a product of incomplete combustion (PIC), or an artifact produced by the MIBAS train.

3.1.1.2 PCDD Results in Study Samples

The PCDD results for study samples are presented in Table 3.3. Very low levels of PCDD were found in the feedstock. The feed waste for this study principally consisted of Aroclor 1260 (about 40 percent (w/w)), with lesser amounts of di-, tri- and tetrachlorobenzenes. The feed waste was mixed in approximately equal portions with No. 2 fuel oil to generate the combustion

TABLE 3.3. LEVELS OF PCDD IN INCINERATION STUDY SAMPLES (NUMBERS IN PARENTHESES INDICATE LEVEL OF DETECTION)

Sample Number	Units	2,3,7,8-Tetra-CDD	Tetra-CDD	Penta-CDD	Hexa-CDD	Hepta-CDD	Octa-CDD
PCB Feed No. 1	ng/g	(0.042)	(0.042)	(0.10)	(0.067)	0.62	4.5
PCB Feed No. 2	ng/g	(0.050)	(0.050)	(0.061)	(0.039)	0.17	4.7
Composite MIBAS No. 1-7	ng/L	0.097	1.2	2.3	12	18	52
Lab Blank	ng/sample	(0.013)	(0.013)	(0.027)	(0.017)	(0.006)	(0.10)

feedstock. The highest PCDD found in the feedstock was approximately 5 ppb (w/w) of octa-CDD and less than 1 ppb (w/w) of the hepta-CDD.

The MIBAS composite sample contained measurable levels for all PCDD congener classes, including 0.097 ng/L of 2,3,7,8-TCDD. The bioassay tests were performed using samples that make up this composite liquid. To determine the exposure of a marine species to PCDD, individual analyses of each of the seven daily samples would have to be determined. Although this composite value could represent only one-seventh of a given daily concentration exposure of marine animals to PCDD, this is highly unlikely to occur.

The laboratory blank was below the measurable detection limit in all cases, indicating no contamination occurred during the laboratory analyses.

3.1.1.3 PCDF Results in Study Samples

PCDF were found at appreciable levels in both the combustion feedstock and the MIBAS composite samples, as shown in Table 3.4. The feedstock was found to have PCDF levels that increased as the level of chlorination increased. Octa-CDF was found at the highest measured levels (2000 and 770 ppb (w/w)), and penta-CDF was found at the lowest level (7.3 ppb (w/w)) for these compound classes. The 2,3,7,8 isomer represents the bulk of the tetrachlorinated congener group.

Some variability is observed between the duplicate feedstock samples for the measurement of higher levels of PCDF chlorination, the hexa-, hepta- and octachlorodibenzofuran. There is no apparent analytical reason for the scatter in these data, which is as high as a factor of 2 to 3 between duplicate analyses. The data clearly indicate increasing concentration with increasing levels of chlorination.

The composite MIBAS seawater sample contained surprisingly high levels of PCDF. These compounds also tend to range upward in concentration as the level of chlorination increases among congener groups. Tetra-CDF was found at 120 pg/ml and octa-CDF was found at 200 pg/ml. Approximately 20 percent of the tetrachlorinated isomers were found as the 2,3,7,8 substituted compound. The laboratory blank was very clean for this analysis, showing only minimal traces of octa-CDF.

TABLE 3.4. LEVELS OF PCDF IN INCINERATION STUDY SAMPLES (NUMBERS IN PARENTHESES INDICATE LEVEL OF DETECTION)

Sample Number	Units	2,3,7,8-Tetra-CDF	Tetra-CDF	Penta-CDF	Hexa-CDF	Hepta-CDF	Octa-CDF
PCB Feed No. 1	ng/g	11	18	7.3(1)	18	680	2000
PCB Feed No. 2	ng/g	12	17	7.3	10	210	770
Composite MIBAS No. 1-7	ng/L	23	120	83	120	150	200
Lab Blank	ng/sample	(0.007)	(0.007)	(0.010)	(0.011)	(0.014)	0.20

(1) Chlorine Isotope Ratio is greater than +/-15 percent of theoretical ratio.

Based on the previously discussed mechanism for conversion of PCB to PCDF, the high levels of PCDF, in most cases 4 to 10 times higher in concentration than the parallel PCDD congeners, was the expected result. These data indicate that pyrocombustive mechanisms and incomplete incineration probably produced the measured PCDF/PCDD found in the MIBAS composite samples. These PCDD/PCDF data appear to follow established patterns for combustion of PCB. The chlorobenzene levels were much more difficult to explain based on the data found in this study.

3.1.1.4 Metals in the Feedstock Samples

An ICAP analysis of the PCB-containing feedstock was performed to determine the background levels of principal inorganic pollutants. If metals were present in the feedstock they would be easier to trace in the combustion control system than organic species. Organic compounds can be converted to different molecular species, but the metals would maintain their chemical identity. For elements that are not found in the materials of construction in the incinerator ductwork, a mass balance could be performed to determine the eventual fate of these discharges. However this was not done.

The metal data are presented in Table 3.5. The PCB feedstock was askarel transformer liquid diluted by half with fuel oil. A 24-element analysis was performed that included principal toxic metals. The screen also included copper, a metal that affects many marine species, and could alter the biological endpoints. The only significant metallic species found in this analysis was iron, which averaged 24 ppm (w/w) in the duplicate analyses. Iron would be expected since PCB-containing liquids were stored in iron vessels. Decomposition of PCB occurs over time, producing HCl; thus some etching would be expected when PCB is stored in iron containers. Only trace levels of calcium, lead, and copper were found in the feedstock. All other metals tested were not detected in the waste feedstock.

TABLE 3.5. METALS ANALYSIS OF PCB FEED STOCK

	Found (ppm)[a]		Detection Limit
Element	PCB Feed 1	PCB Feed 2	(ppm)
Tl	ND	ND	0.25
As	ND	ND	0.25
Se	ND	ND	0.25
Cr	ND	ND	0.05
Sb	ND	ND	0.25
B	ND	ND	0.05
Zn	ND	ND	0.05
Pb	1.5	1.5	0.25
Cd	ND	ND	0.05
Ni	ND	ND	0.10
Ba	ND	ND	0.05
Co	ND	ND	0.05
Mn	0.2	0.1	0.05
Fe	28.5	19.0	0.05
Mg	ND	ND	0.05
Al	ND	ND	0.25
V	ND	ND	0.25
Be	ND	ND	0.05
Ca	0.15	0.40	0.05
Cu	0.10	0.15	0.05
Ag	ND	ND	0.05
Ti	ND	ND	0.10
Na	ND	ND	25.00
K	ND	ND	25.00

(a) ND = Not detected.

3.1.2 Quality Control Samples (Spiked Sampling Trains, MM5 Extract Splits)

A large number of quality control samples were collected in this study in an attempt (1) to completely characterize the flue gas that produced the MIBAS samples and (2) ascertain the performance of the MIBAS train itself. The land-based test was the first time the MIBAS sampling train, with its associated biological and chemical testing, was used to measure the discharge from an operating incinerator. The combusted feedstock was selected to represent a fuel (askarel and fuel oil) similar to the feedstocks proposed for actual marine incineration trial burns. In fact, the quality control samples represent more analyses and more data to interpret than the actual MIBAS composite sample used to measure the potential environmental impact from this flue gas discharge.

The quality control samples are discussed in this section and are separated according to the organic compound class measured. Two gas-phase spiking experiments were performed in this study. Two MM5 trains and two special MIBAS train samples were dosed with the spiked analytes discussed in Section 2.4.1.1. The actual spiking solutions were discussed in Section 2 under sampling procedures. The analytes selected for spiking are the same mixture used to validate the MIBAS train in earlier experiments. Both duplicate spiked samples, the MM5 samples and the MIBAS samples, were collected in a slipstream of flue gas. The sampling location for both spiked runs was selected to represent a flue gas composition equivalent to the composite MIBAS samples used for bioassay tests.

3.1.2.1 PCB Analyses in Quality Control Samples

As shown in Table 3.6, no PCB were detectable at the measured detection limits in any of the spiked MIBAS quality control samples. The spike level corresponded to approximately 3 ng on-column. If one-third of the spiked PCB were found in the seawater catch, (i.e., 30-percent recovery), that amount, 1 ng on-column, would be sufficient for the GC/MS system to measure. The absence of measurable PCB indicates that low recoveries (less than 30

TABLE 3.6. PCB CONCENTRATION FOUND IN QUALITY CONTROL SAMPLE EXTRACTS (NUMBERS IN PARENTHESES INDICATE LEVEL OF DETECTION)

Isomer Group	Amount Found in Sample Extract (µg in Total Sample)							
	Spiked MIBAS Water #1	Spiked MIBAS Water #2	MIBAS XAD-2 Spike #1	MIBAS XAD-2 Spike #2	Spiked MIBAS Organic Rinses #1	Spiked MIBAS Organic Rinses #2	Lab Method Blank, Water	XAD-2 Lab Blank
Monochlorobiphenyl	(0.4)	(0.4)	(0.4)	(0.4)	(0.4)	(0.4)	(0.4)	(0.4)
Dichlorobiphenyl	(0.4)	(0.4)	(0.4)	(0.4)	(0.4)	(0.4)	(0.4)	(0.4)
Trichlorobiphenyl	(0.4)	(0.4)	(0.4)	(0.4)	(0.4)	(0.4)	(0.4)	(0.4)
Tetrachlorobiphenyl	(0.8)	(0.8)	(0.8)	(0.8)	(0.8)	(0.8)	(0.8)	(0.8)
Pentachlorobiphenyl	(0.8)	(0.8)	(0.8)	(0.8)	(0.8)	(0.8)	(0.8)	(0.8)
Hexachlorobiphenyl	(0.8)	(0.8)	(0.8)	(0.8)	(0.8)	(0.8)	(0.8)	(0.8)
Heptachlorobiphenyl	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)
Octachlorobiphenyl	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)
Nonachlorobiphenyl	(1.9)	(1.8)	(1.9)	(1.9)	(1.9)	(1.9)	(1.9)	(1.9)
Decachlorobiphenyl	(1.9)	(1.8)	(1.9)	(1.9)	(1.9)	(1.9)	(1.9)	(1.9)
TOTAL PCB	ND	ND	ND	ND	ND	ND	ND	ND

ND = Not detected.

percent) were found in the MIBAS runs. The recoveries for PCDD, which are described later, indicate that low recoveries in the spiked MIBAS run did indeed occur.

The spiked MM5 runs and the MM5 samples were collected at a point directly opposite the MIBAS sampling location. The results for these samples are presented in Table 3.7. In the spiked MM5 runs, no measurable PCB was found, also indicating that recoveries were below the detection limit of the GC/MS PCB analytical method used.

3.1.2.2 Chlorobenzene Analyses in Quality Control Samples

The spiked MIBAS runs (Table 3.8) were found to contain tri- and tetrachlorobenzenes at a level several times higher than the spike level of approximately 1.6 µg. The trichlorobenzene level determined was 8.8 µg in the first spiked run and 11.8 µg in the second. The tetrachlorobenzene level was 6.0 µg in the first MIBAS spiked experiment and 8.4 in the second. The entire tri- and tetrachlorobenzene catch was found in the XAD resin bed. In the earlier validation program to measure performance of the MIBAS train, high levels of tri- and tetrachlorobenzene were found on the XAD-2 resin bed and none of the spike analytes for this class of compounds were found in the seawater catch or wall rinses. This result indicates that tri- and tetrachlorobenzenes, if present in the combustions gas emissions, will be poorly trapped by the MIBAS train. The results of this spiked experiment confirm the earlier findings.

Since high levels of tri- and tetrachlorobenzene were present in the feedstock, it is impossible to determine the contribution of spiked chlorobenzenes to the native amounts found. The measured levels are several times higher in concentration than the spiked levels. No labeled tri- and tetrachlorobenzene reference spikes were available at the time this spiking experiment was conducted. In future spiked experiments, labeled analytes should be synthesized. Labeled analytes can be measured in the presence of native compounds that arise from incomplete combustion or artifact formation.

TABLE 3.7. PCB CONCENTRATION FOUND IN QUALITY CONTROL SAMPLE EXTRACTS (NUMBERS IN PARENTHESES INDICATE LEVEL OF DETECTION)

Isomer Group	Amount Found in Sample Extract (µg in Total Sample)					
	MM5 Extract No. 1	MM5 Extract No. 2	MM5 Extract No. 3	Spiked MM5 No. 1	Spiked MM5 No. 2	MM5 Blank
Monochlorobiphenyl	(0.4)	(0.4)	(0.4)	(4)	(10)	(4)
Dichlorobiphenyl	(0.4)	(0.4)	(0.4)	(4)	(10)	(4)
Trichlorobiphenyl	(0.4)	(0.4)	(0.4)	(4)	(10)	(4)
Tetrachlorobiphenyl	(0.8)	(0.8)	(0.8)	(8)	(20)	(8)
Pentachlorobiphenyl	(0.8)	(0.8)	(0.8)	(8)	(20)	(8)
Hexachlorobiphenyl	(0.8)	(0.8)	(0.8)	(8)	(20)	(8)
Heptachlorobiphenyl	(1.1)	(1.1)	(1.1)	(11)	(28)	(11)
Octachlorobiphenyl	(1.1)	(1.1)	(1.1)	(11)	(28)	(11)
Nonachlorobiphenyl	(1.9)	(1.9)	(1.9)	(19)	(48)	(19)
Decachlorobiphenyl	(1.9)	(1.9)	(1.9)	(19)	(48)	(19)
TOTAL PCB	ND	ND	ND	ND	ND	ND

ND = Not detected.

TABLE 3.8. CHLOROBENZENE CONCENTRATION FOUND IN QUALITY CONTROL SAMPLE EXTRACTS
(NUMBERS IN PARENTHESES INDICATE LEVEL OF DETECTION)

	Amount Found in Sample Extract (µg in Total Sample)							
Isomer Group	Spiked MIBAS Water #1	Spiked MIBAS Water #2	Spiked MIBAS Organic Rinses #1	Spiked MIBAS Organic Rinses #2	MIBAS XAD-2 Spike #1	MIBAS XAD-2 Spike #2	Lab Method Blank, Water	XAD-2 Lab Blank
Chlorobenzene	(0.4)	(0.4)	(0.4)	(0.4)	(0.4)	(0.4)	(0.4)	(0.4)
Dichlorobenzenes	(0.4)	(0.4)	(0.4)	(0.4)	(0.4)	(0.4)	(0.4)	(0.4)
Trichlorobenzenes	(0.6)	(0.6)	(0.6)	(0.6)	8.8	11.8	(0.6)	(0.6)
Tetrachlorobenzenes	(0.8)	(0.8)	(0.8)	(0.8)	6.0	8.4	(0.8)	(0.8)
Pentachlorobenzenes	(1.0)	(1.0)	(1.0)	(1.0)	(1.0)	(1.0)	(1.0)	(1.0)
Hexachlorobenzene	(1.2)	(1.2)	(1.2)	(1.2)	(1.2)	(1.2)	(1.2)	(1.2)
TOTAL CB	ND	ND	ND	ND	14.8	20.2	ND	ND

ND = Not detected.

Results of chlorobenzene spiking experiments are presented in Table 3.9 for the spiked MM5 runs and the unspiked MM5 samples. The unspiked samples were found to contain a small amount of trichlorobenzene that had passed through the afterburner. Finding higher levels of tri- and tetrachlorobenzene than PCDD/PCDF in MM5 samples may indicate that the MM5 train suffers the same limitation on collecting tri- and tetrachlorobenzenes observed with the MIBAS train. In one spiked MM5 run (Number 1), a high level of tetrachlorobenzene was observed. Because the measured level, 8.0 µg, is much higher than the spiked amount, 1.54 µg, an accurate recovery cannot be estimated based on this measured tetrachlorobenzene level. The OCDD results for these single spiked MIBAS runs, performed on Days 4 and 5 of the test sequence (see Table 2.2) were an order of magnitude lower than levels determined for the composite MIBAS sample (Table 3.3). Note that these spiked MIBAS samples were not included in the composite sample.

3.1.2.3 PCDD Analyses in Quality Control Samples

The results of PCDD analyses in the quality control samples are given in Table 3.10. The spiked MIBAS samples were found to contain very low recoveries of the spiked dioxin recovery standard, 1,2,3,4-tetra-CDD. The total tetra-CDD congener group reported in Table 3.10 for the MIBAS and spiked MM5 runs corresponded to 1,2,3,4-tetra-CDD, the only compound found in the tetra-CDD group. Recoveries presented in Table 3.11 are based on the fact that spiked standard was the only tetra-CDD measured.

The spiked MIBAS train recoveries are given in Table 3.11 and assume that all the measured 1,2,3,4-tetra-CDD came from the spiked recovery standard. These recoveries were disappointingly low, totalling 3.0 percent in Spiked MIBAS Run Number 1, and 6.4 percent in Spiked MIBAS Run Number 2. In the validation program for the MIBAS train (Boehm et al., 1985), the average

TABLE 3.9. CHLOROBENZENES FOUND IN QUALITY CONTROL SAMPLE EXTRACTS (NUMBERS IN PARENTHESES INDICATE LEVEL OF DETECTION)

	Amount Found in Sample Extract (μg in Total Sample)					
Isomer Group	MM5 Extract No. 1	MM5 Extract No. 2	MM5 Extract No. 3	Spiked MM5 No. 1	Spiked MM5 No. 2	MM5 Blank
Chlorobenzene	(0.4)	(0.4)	(0.4)	(4)	(10)	(4)
Dichlorobenzenes	(0.4)	(0.4)	(0.4)	(4)	(10)	(4)
Trichlorobenzenes	1.0	2.0	1.2	(6)	(15)	(6)
Tetrachlorobenzenes	(0.8)	(0.8)	(0.8)	8.0	(20)	(8)
Pentachlorobenzenes	(1.0)	(1.0)	(1.0)	(10)	(25)	(10)
Hexachlorobenzene	(1.2)	(1.2)	(1.2)	(12)	(30)	(12)
TOTAL CB	1.0	2.0	1.2	8.0	ND	ND

ND = Not detected.

TABLE 3.10. LEVELS OF PCDD IN INCINERATION QUALITY CONTROL SAMPLES (NUMBERS IN PARENTHESES INDICATE LEVEL OF DETECTION)

Sample Number	Units	2,3,7,8-Tetra-CDD	Tetra-CDD	Penta-CDD	Hexa-CDD	Hepta-CDD	Octa-CDD
Spiked MIBAS No. 1 Water	ng/sample	(0.085)	2.9[a]	(0.065)	(0.037)	(0.035)	3.8
Spiked MIBAS No. 2 Water	ng/sample	(0.029)	1.1[a]	(0.055)	(0.023)	(0.067)	2.5
Spiked MIBAS No. 1 Rinse	ng/sample	(0.32)	37[a]	0.51	0.056	0.22[b]	1.7
Spiked MIBAS No. 2 Rinse	ng/sample	(0.46)	57[a]	2.8	(0.012)	0.11	0.63
Spiked MIBAS No. 1 XAD	ng/sample	(0.12)	7,6[a]	(0.036)	(0.034)	(0.038)	(0.24)
Spiked MIBAS No. 2 XAD	ng/sample	(0.43)	43[a]	(0.056)	(0.036)	0.10	4.0
Lab Blank	ng/sample	(0.10)	(0.10)	(0.16)	(0.075)	(0.10)	(0.70)
MM5 Extract No. 1	ng/sample	(0.12)	(0.12)	(0.17)	(0.074)	0.17[b]	1.3
MM5 Extract No. 2	ng/sample	0.11	0.11	(0.21)	0.14	0.67	1.5
MM5 Extract No. 3	ng/sample	0.089	0.089	(0.13)	(0.047)	(0.059)	0.44
Spiked MM5 No. 1	ng/sample	(0.64)	390[a]	40	(1.4)	(0.48)	(2.0)
Spiked MM5 No. 2	ng/sample	(2.1)	240[a]	14	5.5	6.6	15

(a) Mass spectral data confirmed that tetra-CDD levels reported here are principally 1,2,3,4-tetra-CDD corresponding to the native spike material.

(b) Chlorine Isotope Ratio is greater than ±15 percent of theoretical ratio.

TABLE 3.11. SPIKED MIBAS TRAIN RECOVERIES

Sample	1,2,3,4-tetra-CDD Percent Recovery
MIBAS Water No. 1(1)	0.18
MIBAS Rinse No. 1(2)	2.3
MIBAS XAD No. 1	0.48
Total	3.0
MIBAS Water No. 2(1)	0.07
MIBAS Rinse No. 2(2)	3.6
MIBAS XAD No. 2	2.7
Total	6.4

(1) Seawater catch and seawater rinses.

(2) Organic solvent (methylene chloride) rinse of glassware in the MIBAS train.

recovery for gas-phase spikes of 1,2,3,4-tetra-CDD in the MIBAS seawater catch was 31 percent (n=7), and the total recovery was 48 percent when the wall rinse and XAD-2 catch were included. The results of this field study are roughly 10 percent of the expected recovery based on the earlier validation results.

The spiked MM5 train produced higher recoveries for the gas-phase spikes of 1,2,3,4-tetra-CDD than the spiked MIBAS run. The measured levels were 390 ng in Run Number 1, and 240 μg in Run Number 2, corresponding to recoveries of 24 percent and 15 percent, respectively. These recoveries are consistent with the expected performance of the gas-phase spiking system and MM5 train.

In the three nonspiked MM5 runs (Table 3.10), the samples collected at sampling port 2, PCDDs were detected at low levels. Octa-CDD was detected in all three runs.

3.1.2.4 PCDF Analyses in Quality Control Samples

The results of PCDF analyses in the quality control samples are shown in Table 3.12. PCDFs were detected in the seawater catch from the spiked MIBAS runs but not in the wall rinse or XAD-2 samples. No PCDF were spiked in this test but the levels of native PCDF were significantly lower in the spiked MIBAS runs than in the MIBAS composite sample used for bioassay testing. The composite sample had native PCDF levels 100 times greater than found in the spiked runs. This difference in measured concentrations could be the result of daily variations in the combustion system, but the low recoveries for 1,2,3,4-tetra-CDD could also indicate an experimental problem with this sample. This possibility will be tested in future studies.

The analysis of native PCDF in the spiked MM5 runs is also provided in Table 3.12. The MM5 runs at sampling port 3 were found to have levels of PCDF that were higher than the observed PCDD concentrations for these samples. Thus, as expected, discharges at the locations sampled contained higher levels of PCDF than PCDD.

TABLE 3.12. LEVELS OF PCDF IN INCINERATION QUALITY CONTROL SAMPLES (NUMBERS IN PARENTHESES INDICATE LEVEL OF DETECTION)

Sample Number	Units	2,3,7,8-Tetra-CDF	Tetra-CDF	Penta-CDF	Hexa-CDF	Hepta-CDF	Octa-CDF
Spiked MIBAS No. 1 Water	ng/sample	0.19	0.52	0.33	0.12	(0.066)	(0.30)
Spiked MIBAS No. 2 Water	ng/sample	0.092	0.19	(0.024)	(0.015)	(0.074)	(0.30)
Spiked MIBAS No. 1 Rinse	ng/sample	(0.055)	(0.055)	(0.011)	(0.020)	0.21(a)	1.1
Spiked MIBAS No. 2 Rinse	ng/sample	(0.036)	(0.036)	(0.011)	0.027	0.071	0.32
Spiked MIBAS No. 1 XAD	ng/sample	(0.026)	(0.026)	(0.018)	(0.016)	(0.039)	(0.057)
Spiked MIBAS No. 2 XAD	ng/sample	(0.027)	(0.027)	(0.018)	(0.021)	(0.025)	(0.23)
Lab Blank	ng/sample	(0.12)	(0.12)	(0.083)	(0.10)	(0.24)	(0.64)
MM5 Extract No. 1	ng/sample	0.24	0.24	(0.040)	(0.046)	(0.097)	(0.33)
MM5 Extract No. 2	ng/sample	0.32	0.42	(0.070)	(0.046)	(0.069)	(0.43)
MM5 Extract No. 3	ng/sample	0.13(a)	0.13(a)	(0.041)	(0.050)	(0.081)	(0.25)
Spiked MM5 No. 1	ng/sample	2.9	7.2	0.33	(0.54)	(0.48)	(1.5)
Spiked MM5 No. 2	ng/sample	41	93	42	25	19	30
Lab Blank	ng/sample	(0.062)	(0.062)	(0.011)	(0.033)	(0.075)	(0.48)

(a)Chlorine Isotope Ratio is greater than +/-15 percent of theoretical ratio.

3.1.3 Emission Levels

It is useful to convert the measured mass of test analytes into emission levels in order to compare these discharges to similar combustion systems. The amounts of flue gas sampled in each sampling system is shown in Table 3.13.

The MIBAS train collects a large sample volume compared to the MM5 train. In the composite MIBAS test, the average sample was approximately 10 DSNm^3, for a combined total of 72.09 DSNm^3 over the seven-day sampling episode. This large volume of gas provides a large sample for bioassay tests. A typical MM5 sample is 2-6 DSNm^3. In the Arkansas tests, the stack MM5 samples ranged from 3.25 to 4.08 DSNm^3, typical collection volumes, but the spiked MM5 samples were 9.0 and 8.6 DSNm^3, respectively.

The PCDD emission results are provided in Table 3.14. The composite MIBAS results are given along with the concentrations of native PCDD in the quality control samples. The composite MIBAS results ranged from 40 pg/DSNm^3 for 2,3,7,8-tetra-CDD to 20 ng/DSNm^3 for octa-CDD. The EPA established an informal target of 1 pg/DSNm^3 as a detection limit for 2,3,7,8-tetra-CDD in ambient air samples. The ambient air sampling technique used by EPA has been validated at 50 pg/DSNm^3. Based on these target levels, the measured MIBAS emissions correspond to low ambient concentrations of PCDD, such as levels found in buildings after a PCB transformer fire.

The emission levels of PCDF are given in Table 3.15. These data show that the furan levels are higher than PCDD levels as expected. The measured PCDF emission for the MIBAS composite sample corresponded to a significant concentration of gas-phase material. The values reported in Table 3.15 for the MIBAS composite sample ranged from 8.9 ng/DSNm^3 for 2,3,7,8-tetra-CDF to 78.0 ng/DSNm^3 for octa-CDF. For comparison, the native PCDF concentrations found in the quality control samples are also included in Table 3.15. The spiked MIBAS samples are shown to have much lower

TABLE 3.13. FLUE GAS SAMPLED

Sample	Type	Collected Volume (DSNm^3)
MIBAS	Day 1(1)	10.03
	Day 2	10.28
	Day 3	11.88
	Day 4	9.89
	Day 5	10.28
	Day 6	9.65
	Day 7	10.11
	Total Composite	72.09
MIBAS	Spiked No. 1	8.88
	Spiked No. 2	9.02
MM5	No. 1	4.08
	No. 2	3.25
	No. 3	3.55
MM5	Spiked No. 1	9.0
	Spiked No. 2	8.6

TABLE 3.14. PCDD EMISSION RESULTS (ng/DSNm^3)

Sample	Type	2,3,7,8-tetra-CDD	Tetra-CDD	Penta-CDD	Hexa-CDD	Hepta-CDD	Octa-CDD
			Study Sample				
MIBAS	Composite	0.04	0.47	0.89	4.7	7.0	20
			Quality Control Samples(a)				
MIBAS(b)	Spiked No. 1	BQL	BQL	BQL	BQL	BQL	0.43
MIBAS(b)	Spiked No. 2	BQL	BQL	BQL	BQL	BQL	0.28
MM5(c)	Spiked No. 1	BQL	43.3(d)	4.44	BQL	BQL	BQL
MM5(c)	Spiked No. 2	BQL	27.9(e)	1.63	0.64	0.77	1.74
MM5	No. 1(f)	BQL	BQL	BQL	BQL	0.04	0.32
MM5	No. 2(f)	0.03	BQL	BQL	0.04	0.21	0.46
MM5	No. 3(f)	0.03	BQL	BQL	BQL	BQL	0.12

(a) BQL = Below quantification limit.

(b) Seawater and seawater rinse sample only. Does not include organic solvent rinse data.

(c) Collected adjacent to the MIBAS sampling location approximately 2 meters past the afterburner chamber.

(d) Represents 24 percent recovery of the native 1,2,3,4-tetra-CDD spike.

(e) Represents 15 percent recovery of the native 1,2,3,4-tetra-CDD spike.

(f) This sample is characteristic of flue gas in the vicinity of MIBAS samples.

TABLE 3.15. PCDF EMISSION RESULTS (ng/DSNm^3)

Sample	Type	2,3,7,8-tetra-CDF	Tetra-CDF	Penta-CDF	Hexa-CDF	Hepta-CDF	Octa-CDF
		Study Sample					
MIBAS	Composite	8.9	47	32	47	58	78
		Quality Control Samples(a)					
MIBAS(b)	Spiked No. 1	0.02	0.06	0.04	0.01	BQL	BQL
MIBAS(b)	Spiked No. 2	0.01	0.02	BQL	BQL	BQL	BQL
MM5(c)	Spiked No. 1	0.32	0.80	0.04	BQL	BQL	BQL
MM5(c)	Spiked No. 2	4.77	10.8	4.8	2.9	2.2	3.5
MM5(d)	No. 1	0.06	0.06	BQL	BQL	BQL	BQL
MM5(d)	No. 2	0.10	0.13	BQL	BQL	BQL	BQL
MM5(d)	No. 3	0.04	0.04	BQL	BQL	BQL	BQL

(a) BQL = below quantification limit.

(b) Seawater and seawater rinse sample only.

(c) Sample collected adjacent to the MIBAS sampling location approximately 2 meters past the afterburner chamber.

(d) This sample is characteristic of flue gas in the vicinity of MIBAS samples.

native PCDF levels than the composite sample. The measured spike recoveries of 1,2,3,4-TCDD were very low for this sample indicating that an operational difficulty may have caused the low PCDF concentrations in the spiked MIBAS runs. This possibility will be investigated in future studies. The spiked MM5 samples revealed one sample with low concentrations of PCDF (Number 1) and one sample with fairly high levels of PCDF (Number 2). The scatter in these two samples point to inherent variability between runs. The three MM5 samples collected in the postcombustion discharge were found to have very low levels of PCDF.

The emission results presented in Tables 3.14 and Table 3.15 were used to calculate destruction efficiencies for the CRF liquid injection incinerator. The following equation was used to calculate these destruction efficiencies (DE):

$$DE = \frac{W_i - F_o C_o}{W_i}$$

where:

W_i = Mass feed rate of analyte into the incinerator (µg/hr)
F_o = Flow rate of afterburner emissions (DSNm^3/min)
C_o = Concentration of analyte in afterburner emissions (ng/DSNm^3).

The destruction efficiencies for spiked MIBAS Runs 1 and 2 are presented in Tables 3.16 and 3.17, respectively, and for spiked MM5 Runs 1 and 2 in Tables 3.18 and 3.19, respectively. For all runs, the destruction efficiencies for total PCB and CB were greater than or equal to 99.9999 percent. Greater than 99.9 percent destruction efficiencies were obtained for all PCDD and PCDF in the spiked MIBAS Runs 1 and 2. For the PCDD and PCDF detected in the emissions from the spiked MM5 Run 1, recoveries ranged from 99.9579 to 99.9953 percent. The destruction efficiencies for PCDD and PCDF in the spiked MM5 Run 2 ranged from 94.4279 to 99.9977 percent.

TABLE 3.16. DESTRUCTION EFFICIENCIES FOR SPIKED MIBAS RUN 1 (3/8/86)

	Amount in Feed (ng/g)[a]	Fuel Feed Rate (Kg/hr)	Mass Feed Rate (μg/hr)	Emission Concentration[b] (ng/DSNm^3)	Gas Flow Rate (DSNm^3/min)	Destruction Efficiency (%)
PCB	244[c]	45	10,980[d]	(0.22)[e]	7.18	>99.9999
CB	138[c]	45	6,210[d]	1.67[e]	7.18	99.9999
Hepta-CDD	0.40	45	18.1	BQL	7.18	>99.9999
Octa-CDD	4.6	45	208	0.43	7.18	99.9122
2,3,7,8-TCDF	12	45	544	0.02	7.18	99.9984
Tetra-CDF	18	45	816	0.06	7.18	99.9968
Penta-CDF	7.3	45	331	0.04	7.18	99.9948
Hexa-CDF	14	45	635	0.01	7.18	99.9993
Hepta-CDF	445	45	20,200	BQL	7.18	>99.9999
Octa-CDF	1385	45	62,800	BQL	7.18	>99.9999

[a] Average results for two feed samples.

[b] Data in parentheses were calculated from the analytical level of detection since compound was not detected in emission samples; for PCB the maximum level of detection for a single isomer group was used.

[c] Data are in g/kg.

[d] Data are in g/hr.

[e] Data are in μg/DSNm^3.

TABLE 3.17. DESTRUCTION EFFICIENCIES FOR SPIKED MIBAS RUN 2 (3/9/86)

	Amount in Feed (ng/g)[a]	Fuel Feed Rate (Kg/hr)	Mass Feed Rate (μg/hr)	Emission Concentration[b] (ng/DSNm^3)	Gas Flow Rate (DSNm^3/min)	Destruction Efficiency (%)
PCB	244[c]	52	12,688[d]	(0.21)[e]	6.92	>99.9999
CB	138[c]	52	7,176[d]	2.2[e]	6.92	99.9999
Hepta-CDD	0.40	52	20.9	BQL	6.92	99.9999
Octa-CDD	4.6	52	240	0.28	6.92	99.9504
2,3,7,8-tetra-CDF	12	52	626	0.01	6.92	99.9993
Tetra-CDF	18	52	939	0.02	6.92	99.9991
Penta-CDF	7.3	52	381	BQL	6.92	>99.9999
Hexa-CDF	14	52	730	BQL	6.92	>99.9999
Hepta-CDF	445	52	23,200	BQL	6.92	>99.9999
Octa-CDF	1385	52	72,200	BQL	6.92	>99.9999

[a] Average results for two feed samples.

[b] Data in parentheses were calculated from the analytical level of detection since compound was not detected in emission samples; for PCB the maximum level of detection for a single isomer group was used.

[c] Data are in g/kg.

[d] Data are in g/hr.

[e] Data are in μg/DSNm^3.

TABLE 3.18. DESTRUCTION EFFICIENCIES FOR SPIKED MM5 RUN 1 (3/6/86)

	Amount in Feed (ng/g)[a]	Fuel Feed Rate (Kg/hr)	Mass Feed Rate (μg/hr)	Emission Concentration[b] ($ng/DSNm^3$)	Gas Flow Rate ($DSNm^3/min$)	Destruction Efficiency (%)
PCB	244[c]	50	12,200[d]	(2.1)[e]	7.16	>99.9999
CB	138[c]	50	6,900[d]	0.89[e]	7.16	99.9999
Hepta-CDD	0.40	50	20.0	(0.053)	7.16	>99.8862
Octa-CDD	4.6	50	230	(0.22)	7.16	>99.9589
2,3,7,8-Tetra-CDF	12	50	599	0.32	7.16	99.9770
Tetra-CDF	18	50	898	0.88	7.16	99.9579
Penta-CDF	7.3	50	364	0.04	7.16	99.9953
Hexa-CDF	14	50	699	(0.060)	7.16	>99.9963
Hepta-CDF	445	50	22,200	(0.053)	7.16	>99.9999
Octa-CDF	1385	50	69,100	(0.17)	7.16	>99.9999

[a] Average results for two feed samples.

[b] Data in parentheses were calculated from the analytical level of detection since compound was not detected in emission samples; for PCB the maximum level of detection for a single isomer group was used.

[c] Data are in g/kg.

[d] Data are in g/hr.

[e] Data are in $μg/DSNm^3$.

TABLE 3.19. DESTRUCTION EFFICIENCIES FOR SPIKED MM5 RUN 2 (3/11/86)

	Amount in Feed (ng/g)[a]	Fuel Feed Rate (Kg/hr)	Mass Feed Rate (μg/hr)	Emission Concentration[b] (ng/DSNm^3)	Gas Flow Rate (DSNm^3/min)	Destruction Efficiency (%)
PCB	244[c]	45	10,980[d]	(5.6)[e]	7.02	>99.9999
CB	138[c]	45	6,210[d]	(3.5)[e]	7.02	>99.9999
Hepta-CDD	0.40	45	18.1	0.77	7.02	98.2090
Octa-CDD	4.6	45	209	1.74	7.02	99.6495
2,3,7,8-Tetra-CDF	12	45	544	4.77	7.02	99.6309
Tetra-CDF	18	45	816	10.8	7.02	94.4279
Penta-CDF	7.3	45	331	4.8	7.02	99.3895
Hexa-CDF	14	45	635	2.9	7.02	99.8077
Hepta-CDF	445	45	20,200	2.2	7.02	99.9954
Octa-CDF	1385	45	62,800	3.5	7.02	99.9977

[a] Average results for two feed samples.

[b] Data in parentheses were calculated from the analytical level of detection since compound was not detected in emission samples; for PCB the maximum level of detection for a single isomer group was used.

[c] Data are in g/kg.

[d] Data are in g/hr.

[e] Data are in μg/DSNm^3.

A comparison of the amount of emissions generated by the liquid injection incinerator at the CRF with those of the incinerator on the Vulcanus is presented in Table 3.20. The Vulcanus data are assumed as one stack. These data were obtained from Ackerman, et al. (1978) and VanderVelde and Glod (1986), except for the firing rate which was calculated assuming the same feed material as used with the CRF, and the emission rate which is applied from the CRF data. All data for the CRF are from Lee, et al. (1987) and are averaged data when more than one value is reported. The data are comparable in that the ratio of stack flow to air input flow is approximately the same for the two incinerators (53 and 58) and the ratio of the waste feed rate to the firing rate is similar (120 and 122). Therefore, a direct extrapolation of the average PCB emission rate of 0.2 $\mu g/m^3$ is considered a good estimate of the potential Vulcanus emission rate and the resulting total emission value of 39.6 mg of PCB for three hours of test operation for one stack of the Vulcanus.

3.1.4 Mass Spectral Analysis (GC/MS Scans)

In addition to the analysis of PCB, chlorobenzene, PCDD, and PCDF, the mass spectral fragmentation patterns were searched by a computer "best fit" program that assigned possible structures to the principal peaks observed in the composite MIBAS sample and the three stack MM5 extracts. The results of the composite MIBAS sample analysis are given in Appendix C, along with the results from the three stack MM5 samples. The general observations from these data are that fatty acid methyl esters, hydrocarbons, substituted benzene, and cyclohexane derivatives are the principal constituents. The cyclohexane and substituted cyclohexane data should be considered suspect because cyclohexane is added to methylene chloride as a preservative and will produce cyclohexyl products in the presence of reactive extracts.

TABLE 3.20. EXTRAPOLATION OF CRF PCB EMISSION LEVELS TO *Vulcanus* I LEVELS

	Vulcanus	CRF
Stack Flow (m^3/hr)	66,000	1240
Waste Feed (kg/hr)	6,000	50
Air Input (m^3/hr)	30,000	520
Chamber Temperature (°C, min)	1,250	1180[a]
Firing Rate (GJ/hr)	196[b]	1.6
PCB Emission Rate ($\mu g/m^3$)	0.2[d]	0.2[c]
Total Emission (mg/3 hrs)	39.6[d]	0.74

[a] Average of maximum and minimum values.

[b] Calculated based on average fuel HHV from CRF.

[c] Average of three MM5 tests.

[d] Assumed.

3.1.5 Analytical Quality Assurance

Quality assurance activities conducted to support the chemical analyses included the following:

- Collection and analysis of quality control samples
- Collection and analysis of laboratory blanks
- Determination of laboratory spike recoveries.

3.1.5.1 Collection and Analysis of Quality Control Samples

The quality control samples collected in this program included the two spiked MIBAS samples and the two spiked MM5 samples. As previously described, these samples were collected by spiking the incinerator emissions with PCB, chlorobenzene, and PCDD compounds; and sampling the spiked emissions with the MIBAS or MM5 train. The compounds spiked into the emissions in each test included:

1,2,4-trichlorobenzene
1,2,4,5-tetrachlorobenzene
pyrene
3,3',4,4'-tetrachlorobiphenyl
chrysene
2,2',3,3',5,5',6,6'-octachlorobiphenyl
pentachlorophenol
1,2,3,4-tetrachlorodibenzo-p-dioxin.

Analysis of these compounds in the quality control samples demonstrates the collection efficiency of the sampling train and the effective recovery of PCB, chlorobenzene, and PCDD/PCDF compounds through sampling and analysis.

The results of the analysis of the quality control samples are previously presented in Tables 3.6 through 3.12. The two PCB compounds spiked into the emissions were not detected in any of the four quality control samples.

Trichlorobenzenes and tetrachlorobenzenes were detected in the spiked MIBAS and spiked MM5 samples; however, spike recoveries could not be calculated since native chlorobenzenes in the emissions were also contributing to the chlorobenzene levels detected. Recoveries of the 1,2,3,4-tetra-CDD spiked into the MIBAS samples were 3.0 percent in Run 1, and 6.4 percent in Run 2. Higher recoveries of 24 percent and 15 percent for Run 1 and Run 2, respectively, were obtained with the MM5 train for 1,2,3,4-tetra-CDD.

The low spike recoveries obtained for these samples could have been caused by several factors: (1) the gas-phase spiking system may not have effectively delivered the spike compounds into the emission stream; (2) the MIBAS may not have efficiently collected the compounds from the emissions; or (3) the compounds may have been lost from the samples during shipping, preparation, or analysis. Since the performance of the gas-phase spiking system and the MIBAS train have been demonstrated in previous studies, and the analytical procedures used have been validated, identifying one of these factors as the single cause of the low recoveries is difficult. Most likely, a combination of these and other factors contributed to the low recoveries.

3.1.5.2 Preparation and Analysis of Laboratory Blanks

Various laboratory blanks were prepared and analyzed in this study. These blanks are used to demonstrate that samples are not exposed to possible sources of contamination in laboratory activities.

The laboratory blanks analyzed in this program included the following:

- Composite MIBAS laboratory blank
- Spiked MIBAS water laboratory blank
- Spiked MIBAS XAD-2 laboratory blank
- MM5 laboratory blank.

All blanks were treated and processed in the same manner as the associated samples.

In the laboratory blank processed with the composite MIBAS samples, no PCDD were detected. Of the PCDF, only 0.20 ng of octa-CDF was detected in this blank. This level is insignificant when compared with the levels of PCDF found in the composite MIBAS sample (23 to 200 ng/L). Consequently, PCDD/PCDF contamination of the composite MIBAS sample from laboratory activities is not expected to have occurred.

No PCB, chlorobenzenes, or PCDD/PCDF were detected in the laboratory method blank processed with the MM5 samples and the water and XAD-2 laboratory blanks processed with the spiked MIBAS samples. These results indicate that the spiked MIBAS and MM5 samples were probably not exposed to contamination in the laboratory.

3.5.1.3 Determination of Laboratory Recovery Spikes

As described in Section 2, each sample was spiked with labeled compounds prior to extraction or preparation. Determination of the recoveries of these labeled compounds indicate the efficiency of the extraction and preparation procedures for recovering native analytes of interest.

The following labeled compounds spiked in each sample were analyzed: 2,3,7,8-tetra-CDF-$^{13}C_{12}$, 2,3,7,8-tetra-CDD-$^{13}C_{12}$, and octa-CDD-$^{13}C_{12}$. Recoveries of these labeled compounds are presented in Table 3.21.

As shown, recoveries of the labeled compounds were above 50 percent for the composite MIBAS and spiked MIBAS samples, except for the recoveries of octa-CDD from the two spiked MIBAS samples. The low recoveries obtained in these two cases (19 and 7 percent) are unusual. For the PCB feed samples, good recoveries were obtained for 2,3,7,8-tetra-CDD-$^{13}C_{12}$ (95 percent for both samples), while the recoveries for the other two labeled compounds were variable. The recoveries of the labeled compounds from the MM5 extracts prepared by Acurex were uniformly low (less than 23 percent). Since Battelle did not spike or extract these samples, these low recoveries cannot be

TABLE 3.21. RECOVERIES OF LABORATORY SPIKES

Sample Number	2,3,7,8-Tetra-CDF-$^{13}C_{12}$	2,3,7,8-Tetra-CDD-$^{13}C_{12}$	Octa-CDD-$^{13}C_{12}$
PCB Feed No. 1	32	95	13
PCB Feed No. 2	56	95	20
Composite MIBAS No. 1-7	107	101	173
Lab Blank	73	78	124
Spiked MIBAS No. 1 Water	70	82	19
Spiked MIBAS No. 2 Water	58	72	7
Spiked MIBAS No. 1 Rinse	82	91	106
Spiked MIBAS No. 2 Rinse	66	74	109
Spiked MIBAS No. 1 XAD	68	72	52
Spiked MIBAS No. 2 XAD	66	68	79
Lab Blank	85	96	41
MM5 Extract No. 1	21	20	17
MM5 Extract No. 2	21	20	23
MM5 Extract No. 3	21	18	17
Spiked MM5 No. 1	51	51	25
Spiked MM5 No. 2	40	44	33
Lab Blank	52	65	45

explained. The recoveries from the spiked MM5 samples ranged from 25 to 51 percent. Acceptable recoveries were obtained for all laboratory method blanks. These recoveries indicate, that with few exceptions, native PCDD/PCDF were probably efficiently recovered from these samples.

3.2 TOXICITY TEST RESULTS

3.2.1 Water Quality Adjustment Results

Table 3.22 presents a summary of the water quality adjustment data for the MIBAS samples generated during the March 5-11, 1986, land-based tests. The initial volume of the unadjusted subsample was always 1 liter. When the water quality adjustments were completed, the final volume of the water quality-adjusted solution was ten liters. The ionic strength and the pH of the unadjusted MIBAS sample ranged from 96 to $120^{o}/oo$ and 0.00 to 0.09, respectively.

3.2.2 Toxicity Tests

3.2.2.1 *Menidia beryllina*

The results of the seven-day fish test are summarized in Table 3.23. Significant differences from both controls were noted in the two highest concentrations. There was a significant effect on survival in the 3.2 percent concentration, but not on the growth of the survivors. Both survival and growth were affected in the 10 percent concentration; however, only two individuals survived to be used in the determination of mean weight. Therefore, this value may contain a high degree of error. Nearly all of the mortality occurred during the first day of testing.

An additional "mini test" was begun on Day 4 to test whether this sample was more toxic than those obtained during the previous days. Previously unexposed fish were exposed either to the 10 percent MIBAS sample or NAIS water (adjusted to $30^{o}/oo$ salinity). There was only one replicate per treatment. This test was continued with daily renewal for 96 hours. There was no mortality in either treatment during this test. Because this was an acute test with only one replicate per treatment, growth measurements were not made.

TABLE 3.22. WATER QUALITY ADJUSTMENT DATA FOR MIBAS SAMPLES COLLECTED DURING THE PERIOD OF MARCH 5-11, 1986, AT THE EPA COMBUSTION RESEARCH FACILITY IN JEFFERSON, ARKANSAS.

March 1985 Date	Arkansas MIBAS Sample No.	Initial Salinity (⁰/oo)	Initial pH	Volume DI H_2O Added (ml)	Coloration	Approximate Vol. Seawater (30 ⁰/oo) Added (ml)	NaOH (ml) Vol. of pH Adjusting 10N	Final Sample Medium 1.0N	Salinity (⁰/oo)	Final Sample pH
5	1	112	0.09	3000	None	5512	186	1.75	30	7.99
6	2	100	0.00	2850	None	6990	160	--	30	8.02
7	3	120	0.02	3608	yellow/green(a)	6184	204.6	1.9	30	7.98
8	4	96	0.05	2690	None	7157	153.5	--	29	8.03
9	5	120	0.00	3608	light yellow	6170	222	--	30	7.97
10	6	110	0.00	3237	light green	6589	174	--	29.5	8.01
11	7	104	0.00	2860	light green	7128	161.5	0.5	30	8.05

(a)Sample turned amber-colored during pH adjustment. An orange/red precipitate formed.

TABLE 3.23. RESULTS OF THE SEVEN-DAY EXPOSURE OF *Menidia beryllina* TO MIBAS SAMPLES.

	TREATMENT						
Biological Response	**Brine Control**	**Site Control**	**0.1 % MIBAS**	**0.32% MIBAS**	**1.0% MIBAS**	**3.2% MIBAS**	**10% MIBAS**
% Survival	91	95	95	96	93	76*	4*
± SD	±2.8	±3.3	±3.3	±6.1	±5.3	±6.1	±6.1
Mean dry wt.	0.495	0.475	0.524	0.521	0.487	0.428	0.216**
(mg) ± SD	±0.012	±0.024	±0.055	±0.029	±0.09	±0.005	
Mean Temp. °C	24.8	24.7	25.1	25.2	25.2	25.3	25.1
± SD	±0.6	±0.5	±0.6	±0.5	±0.5	±0.6	±0.5
Mean Salinity(a)	34	35	34	33	33	34	34
(°/oo) ± SD	±1	±3	±2	±2	±2	±2	±2
Mean DO (mg/L)	6.4	6.5	6.5	6.5	6.5	6.6	6.8
± SD	±0.6	±0.4	±0.4	±0.4	±0.4	±0.5	±0.4

*Significantly different from controls at an alpha level of 0.05.

**Significantly different from controls at an alpha level of 0.05, however, n=2.

[a] High values due to rapid evaporation.

3.2.2.2 Mysidopsis bahia

The results of the crustacean test are summarized in Table 3.24. As with the fish test, there was a significant decrease in survival in the 3.2 and 10 percent concentrations. Growth was not significantly affected at any concentration in which there were survivors.

Unfortunately, there was a delay (nearly 48 hours) between the termination of the test and the observation of egg production. This delay apparently allowed for the initiation of egg production after the end of the test, as indicated by eggs in the oviducts alone. This renewed production was reflected in the values obtained for the total percentage of gravid females (in the brood sac and/or the oviducts). As a result, this measurement is invalid. However, a delay in egg production is reflected as a substantial decrease in the percentage of total females with eggs in the brood sac (number of females with eggs in the brood sac/total number of females x 100) in the 3.2 percent concentration. This measurement is probably more indicative of the potential effects on egg production than is the presence of eggs in the oviduct, because eggs present in the brood sac were probably produced during the test period. In any event, the delay in making the observations may have increased the variability thereby masking the statistical significance.

As with the fish, the majority of the mortality occurred early in the test.

3.2.2.3 Champia parvula

The macroalga test used in this study was not successful. Apparently the cultures themselves were affected by some undetermined factor (e.g., inadequate light, and/or uninterrupted continuous lighting). Sexual reproduction was nearly absent, even in the controls. Therefore, it was impossible to obtain meaningful results from any of the three tests. The only useful observations were those on tissue necrosis and the general health of the male and female tips after the two-day exposure period; however, this information is very subjective.

TABLE 3.24. RESULTS OF THE SEVEN-DAY EXPOSURE OF *Mysidopsis bahia* TO MIBAS SAMPLES.

Biological Response	TREATMENT				
	Site Control	**0.1% MIBAS**	**1.0% MIBAS**	**3.2% MIBAS**	**10% MIBAS**
% Survival ± SD	85 ±13	80 ±10	83 ±21	35* ±22	0*
Mean dry wt. (mg) ± SD	0.33 ±0.04	0.36 ±0.08	0.29 ±0.06	0.32 ±0.03	--
Mean % females with eggs in brood sac ± SD	30 ±34	31 ±34	14 ±35	0	--
Mean % gravid females ± SD	39 ±42	61 ±41	64 ±44	90 ±20	--
Mean Temp. °C ± SD	25.8 ±0.6	26.2 ±0.6	26.1 ±0.6	26.1 ±0.6	26.6 ±0.6
Mean Salinity(a) (°/oo) ± SD	34 ±1	34 ±1	33 ±1	33 ±2	33 ±2
Mean DO (mg/L) ± SD	5.6 ±0.3	5.6 ±0.2	5.6 ±0.3	6.0 ±0.3	6.0 ±0.3

*Significantly different from site controls at an alpha level of 0.05.

[a] High values due to rapid evaporation.

Because of the failure of this test in the field, aliquots of MIBAS Samples 1, 3, and 5 were brought back to ERL-N and were retested with *C. parvula*. Both a Narragansett Bay and North Atlantic Site control were used. Unlike the tests conducted in Arkansas, Narragansett Bay water was used for the dilutions, and there were only two replicates per treatment. The results of this test are summarized in Table 3.25.

MIBAS 1 (Test 1) appeared to be the most toxic. Plants exposed to concentrations of 1.0 percent and above died during the two-day exposure period. Sexual reproduction was depressed in all concentrations (number of cystocarps being less than 5 percent of control cystocarp production).

MIBAS 3 (Test 2) was the least toxic of the samples tested. Plants exposed to 10 percent MIBAS survived. However, necrotic tissue was observed. Sexual reproduction was depressed at concentrations of 0.32 percent and above.

The toxicity of MIBAS 5 (Test 3) was only slightly less than that of MIBAS 1. Plants exposed to the two highest concentrations died, and those exposed to 1.0 percent suffered severe tissue necrosis. Sexual reproduction was depressed in the 0.32 percent concentration. Cystocarp production was depressed, but not significantly, in the 0.1 percent concentration, however, there was only one replicate for this treatment.

3.2.2.4 Arbacia punctulata

The criteria for accepting or rejecting a replicate test for a particular sample were modified slightly from the original protocol in that tests in which control fertilization exceeded 90 percent were also considered acceptable if a dose response was observed. When control fertilization exceeds 90 percent, the sensitivity of the test may be reduced. Generally, the NAIS controls were given priority in interpreting acceptability. In some cases, however, NAIS control data were not available and the reconstituted brine controls were used to assess the test acceptability.

Table 3.26 summarizes the results for each MIBAS sample. The EC50s ranged from 0.089 to 0.322 percent MIBAS, except for the test with MIBAS 7, in which there was an apparent loss in sensitivity due to high control

TABLE 3.25. RESULTS OF THE *Champia parvula* TESTS CONDUCTED AT ERL-N WITH MIBAS SAMPLES 1, 3, and 5.

Concentration	MEAN NUMBER CYSTOCARPS PRODUCED PER PLANT[a] MIBAS 1	MIBAS 3	MIBAS 5
Narr. Control(b)	15	15	15
Site Control(b)	12	12	12
0.1% MIBAS	0.1(c)	2.2	1.3
0.32% MIBAS	0(c)	0.7(c)	0(c)
1.0% MIBAS	dead	0(c)	SNT(c)
3.2% MIBAS	dead	0(c)	dead
10% MIBAS	dead	SNT(c)	dead

(a)SNT = severe necrotic tissue, no cystocarp production.
(b)Only one of each control was run for all 3 tests.
(c)Cystocarp production less than 5 percent of control cystocarp production.

TABLE 3.26. RESULTS OF SPERM CELL TEST (*Arbacia punctulata*) WITH MIBAS SAMPLES DURING THE PERIOD FROM MARCH 5-11, 1986

Sample Designation	Sample Generation Date	Toxicity Test Date	% Fertilization Brine Controls $\bar{x}$ ±SD	% Fertilization Site Water Controls $\bar{x}$ ±SD	n(a)	% MIBAS Test Range	EC50 (% MIBAS)	95% Confidence Limits	EC50 Calculation Method
MIBAS 1	3/5/86	3/5/86	--	--	--	0.62-10.0	None(b)	--	--
MIBAS 1 (repeated)	3/5/86	3/7/86	98.6 ±1.0	92.9 ±10.8	3	0.062-1.0	0.138	0.132-0.145	moving average
MIBAS 2	3/6/86	3/6/86	--	--	--	0.62-10.0	--(c)	--	--
MIBAS 2 (repeated)	3/6/86	3/8/86	98.8 ±1.4	<50%(d)	4	0.062-1.0	0.303	0.283-0.324	moving average
MIBAS 3	3/7/86	3/7/86	96.7 ±1.2	79.8 ±13.9	3	0.062-1.0	0.089	0.084-0.095	probit
MIBAS 4	3/8/86	3/8/86	99.3 ±0.9	<50%(d)	2	0.062-1.0	0.263	0.242-0.284	moving average
MIBAS 5	3/9/86	3/9/86	97.8 ±1.3	75.8 ±21.8	2	0.062-1.0	0.276	0.253-0.300	moving average
MIBAS 6	3/10/86	3/10/66	95.2 ±2.2	82.2 ±19.3	3	0.062-1.0	0.322	0.300-0.340	moving average
MIBAS 7	3/11/86	3/11/86	97.2	97.8	1	0.062-1.0	0.821	0.5-1.0	binomial

(a) n = number of tests considered acceptable.
(b) Test invalid. Few fertilized eggs observed in controls.
(c) Test invalid. >50% unfertilized in all treatments.
(d) Site water controls apparently contaminated.

fertilization. A description of the daily events relevant to the sperm cell test follows:

March 5, 1986 1. Tested MIBAS 1, which was generated on the same date. The test was invalid due to poor fertilization in the control and treatment exposures. A calibration test series to determine a sperm density in the control exposures which would yield 60-90 percent fertilization was planned. Sperm densities, based on absorbance, that had yielded successful results in trial runs at Battelle in Duxbury did not yield similar results in Arkansas. Sperm and egg collection was difficult in that few animals released gametes when stimulated. Those that did release gametes provided only a weak stream.

March 6, 1986 1. Calibration tests were conducted and it was determined that a sperm density of 0.3 AU (Absorbance Units) would yield acceptable control fertilization.

2. Tested MIBAS 2 generated on March 6. Acceptable control fertilization was observed in two replicate tests at a sperm density of 0.3 AU. However, fertilization in all treatments was less than 50 percent, and, therefore, an EC50 could not be calculated. The exposure concentration series (0.62-10.0 percent MIBAS) was too high.

March 7, 1986 1. The sperm cell test for MIBAS 1 was repeated at an exposure concentration range of 0.062-1.0 percent MIBAS with a sperm density of 0.2 AU. One replicate test yielded fertilization within the range of 60-90 percent. However, two additional test replicates were considered acceptable when control fertilization exceeded 90 percent because these tests were observed to be even more sensitive and, therefore, test insensitivity was not apparent.

2. Tested MIBAS 3 generated on the same date with a sperm density of 0.2 AU. Two replicate tests were acceptable based upon the protocol criteria of 60-90 percent control fertilization. In a third test, the fertilization exceeded 90 percent but the dose-response was similar to the other two tests and test insensitivity was not apparent.

March 8, 1986 1. The test for MIBAS 2 was repeated at sperm densities of 0.2 AU (two replicate tests) and 0.1 AU (two replicate tests) for the exposure range of 0.062-1.0 percent MIBAS. The sperm density was reduced in two replicates in an attempt to lower control fertilization below 90 percent. An unidentified source of contamination resulted in poor fertilization response in the site water controls. Fertilization in the brine controls was greater than 90 percent, though a masking of effect may have occurred in two replicate tests. A dose-response was observed for all four test replicates. They were considered acceptable and the counts were pooled for EC50 analysis.

2. MIBAS 4 was tested simultaneously with the repeat test for MIBAS 2. The same control series and sperm densities were used. The site water controls were rejected due to insufficient fertilization, as described above. Two replicate tests yielded dose-response data which was used for EC50 analysis.

March 9, 1986 1. MIBAS 5 was tested at a sperm density of 0.1 AU. Two replicate tests were considered acceptable based upon control fertilization of 90.8 and 60.7 percent, respectively. The data for the 0.062 percent exposure concentration were deleted from the EC50 analysis because of anomalous results at this exposure level in one replicate test.

March 10, 1986 1. MIBAS 6 was tested at a sperm density of 0.1 AU. Three replicate tests were considered to be acceptable, although control fertilization was greater than 90 percent in two replicate tests. These tests yielded dose-response data although a masking of effect may have occurred in two of the test replicates.

March 11, 1986 1. MIBAS 7 was tested at a sperm density of 0.1 AU. Fertilization in the controls was greater than 90 percent for all replicate tests. Three replicate tests were rejected because no toxic response was observed. One replicate test was accepted because it yielded dose-response data. Test insensitivity was evident for all replicates.

During the seven-day testing period, it became apparent that gamete abundance and probably viability improved throughout the period. With each day it was easier to find animals which would supply gametes and with each day the abundance seemed to increase. It is possible that travel-related stress and acclimation to the static aquarium system during the initial days affected gamete production. Additionally, it is possible that stimulation of some animals followed by their return to the aquarium with some attached residual gametes may have stimulated hormonal responses and a "communal" enhancement of reproductive state.

3.2.2.5 Dinophilus gyrociliatus

The four- and seven-day LC50s were 1.7 and 1.4 percent MIBAS, respectively. There was little change in the water quality parameters between renewals (Table 3.27). Reproduction was significantly decreased at the lowest concentration (0.5 percent MIBAS) tested (Table 3.28). Reproductive inhibition was observed at concentrations well below those that significantly affected survival. Although growth was not measured during this study, obvious growth retardation was observed in all of the MIBAS treatments as compared with the controls.

The reproductive success of the control group was less than normal. The cabinet in which the experiment was conducted was located directly above a fluorescent light fixture, which increased the ambient temperature of the experimental environment above the recommended range when the light was on. Elevated temperatures have been shown to significantly reduce the reproductive success of *D. gyrociliatus* (Carr et al., 1986).

TABLE 3.27. MEAN VALUES FOR WATER QUALITY DATA FOR *Dinophilus gyrociliatus* EXPOSURE MEDIA FOR COMPOSITE SAMPLES FROM EACH TREATMENT ON DAYS 1 AND 6.

Treatment (% MIBAS)	Fresh Sample				24-Hour Old Sample			
	Temp. (°C)	Salinity (°/oo)	DO (% Saturation)	pH	Temp (°C)	Salinity (°/oo)	DO (% Saturation)	pH
Control	21	25	100	7.83	22	25	100	7.79
0.5	21	25	99	7.85	22	25	100	7.77
1.0	21	25	97	7.88	22	25	99	7.77
2.1	21	25	95	7.92	22	25	98	7.77
4.2(a)	22	25	96	7.85	22	25	97	7.69
8.3(a)	22	25	91	7.97	22	25	91	7.58

[a] Values for Day 1 only.

TABLE 3.28. SURVIVAL AND REPRODUCTIVE DATA FOR *Dinophilus gyrociliatus* AT THE TERMINATION OF THE SEVEN-DAY TEST.

Treatment (% MIBAS)	% Survival	Total Number of Eggs Laid	Mean Number of Eggs per Surviving Female (± SE)[a]
Control	86	16	2.7 ± 0.5
0.5	82	14	1.5 ± 0.3[b]
1.0	79	0	0.4 ± 0.1[c]
2.1	38	0	0
4.2	0	0	--

[a] Includes eggs laid and eggs visible in the coelom

[b] Significantly different than control ($p<0.05$)

[c] Significantly different than control ($p<0.001$)

4.0 DISCUSSION

4.1 TOXICITY TEST

The results of this study indicate that toxic effects are observed at concentrations as low as 0.1 percent MIBAS for both animals and plants. As no toxicity was observed at 2 percent MIBAS concentrations in the Level 1 tests with HCl-spiked fuel oil combustion (FOC) samples (Boehm et al., 1985), it appears that there were certainly some toxic components in the Level 2 PCB-MIBAS samples that were not present in the FOC-MIBAS samples.

Of the five tests that were evaluated, the sperm cell test with *Arbacia punctulata* and the macroalgae reproduction test with *Champia parvula* appear to be the most sensitive to the PCB-MIBAS samples (Table 4.1). The life-cycle test with *Dinophilus gyrociliatus* was the most sensitive of the three seven-day chronic tests evaluated. The fish and the mysid tests appear to be the least sensitive to the effects of the Level 2 PCB-MIBAS samples. For two of the three most sensitive species tested (i.e., *Champia parvula* and *Dinophilus gyrociliatus*), an NOEC (no observed effect concentration) was not established because significant effects were observed at the lowest MIBAS concentration tested.

The problem previously encountered with the deionized water used to dilute the reconstituted brine used as dilution water during the Level 1 tests has been resolved with the system used in the new Mobile Lab. Elevated test salinities due to excessive evaporation occurred in the fish and mysid experiments. The following recommendations would help alleviate the problems encountered.

1. The air conditioning/heating system for the lab should be modified to incorporate an activated charcoal filter. This should remove any vapors that may be potentially harmful to the researchers and to the *Champia*.
2. The light intensity for the *Champia* cultures and test chambers needs to be increased, and the lights put on a timer.

TABLE 4.1 SUMMARY OF TEST RESULTS FOR TOXICITY TESTS WITH MIBAS SAMPLES CONDUCTED AT EPA COMBUSTION RESEARCH FACILITY, JEFFERSON, ARKANSAS.

Test Species	Endpoint	LOEC[a] (Percent MIBAS)	NOEC[b] (Percent MIBAS)
Menidia beryllina	Survival	3.2	1.0
	Growth	10	3.2
Mysidopsis bahia	Survival	3.2	1.0
	Growth	(c)	3.2
	Reproduction	(c)	(c)
Champia parvula[d]	Survival	1.0	0.32
	Reproduction	0.1	<0.1
Arbacia punctulata	EC50[e] (percent fertilization)	0.089 to 0.322	0.062 to 0.125
Dinophilus gyrociliatus	Survival	2.1	1.0
	Reproduction	0.5	<0.5

---

[a] Lowest observed effect concentration

[b] No observed effect concentration

[c] Insufficient data

[d] Test performed at ERL-Narragansett

[e] Range of values for MIBAS samples 1-6

3. During transport and holding in the lab, the density of mysids should be reduced to prevent cannibalism. This step will help to assure an adequate number of test animals for future studies.

4. The presence of eggs in mysid oviducts and brood sacs should be determined in the field immediately upon the termination of the test. Any delay may invalidate this measurement.

5. The Dinophilus test should be maintained in a location where the temperature can be more accurately controlled.

6. The exposure chambers for the fish and mysid tests should be equipped with covers to minimize evaporation.

4.2 EMISSION TEST ANALYSES

The results of analyzing both the MIBAS composite sample and the associated quality control samples indicated that significant PCDF were present in the samples used for bioassay testing. PCDD were found in the MIBAS composite sample at lower concentrations than PCDF. This difference in concentration was the expected result for pyrocombustive decomposition of PCB.

In spiked MIBAS runs, the recoveries of synthetic analytes spiked into the train were very poor. The only measurable spike was 1,2,3,4-TCDD at a total recovery of a few percent in each test. No experimental parameter could be identified to explain these low recoveries. In the spiked MM5 experiments, low recoveries of 1,2,3,4-TCDD were found (15 and 24 percent), but this recovery level is consistent with earlier validation programs on the MIBAS train. Since the gas-phase spiking experiment using the MIBAS train has been successfully conducted in pilot plant studies (Boehm et al., 1985), the field operation of this important quality control step should be verified before bioassays are repeated, either for additional land-based tests, or during actual marine incineration. This quality control parameter, spike recovery, is critical to the defensibility of marine impact data measured using the MIBAS system.

Additional problems were identified from the quality control sample analyses in this program. The PCB analytical method may not have the sensitivity to measure PCB or chlorobenzene spiked compounds in experiments where low recoveries (<30 percent) occur. In the future, and especially for marine water samples, higher spike levels, labeled spikes, and sample extracts concentrated to the minimum injection volume possible, will be required to increase sensitivity of the low resolution GC/MS technique. Alternatively, the more sensitive, yet less selective and definitive, gas chromatography-electron capture detection (GC/ECD) may have to be relied on more heavily for spiking experiments and for actual MIBAS sample determinations.

Also, it appeared from the bioassay results that additional toxic analytes could be present in the MIBAS water samples; analytes other than PCDD/PCDF/PCB or chlorobenzenes. These new marine toxicants should be investigated using selective chemical analysis techniques such as HRGC coupled to Fourier Transform Infrared Analysis, or high performance liquid chromatography (HPLC) coupled to Nuclear Magnetic Resonance Spectroscopy Analysis. These products of incomplete combustion (PICs) should be identified as soon as possible so analysis of new compounds can be added to future studies.

The unspiked MM5 samples revealed very low levels of all analytes tested, indicating a very clean stack discharge. Likewise, the analysis of metals in the incineration feedstocks found a fairly clean liquid. The metal analysis identified iron as the only significant metallic element in the fuel feed.

4.3 OVERALL DISCUSSION

The land-based incineration tests performed and reported herein were not as successful as would have been expected from the results obtained on pilot studies conducted previously using the same apparatus and techniques. Recovery of spiked compounds in the spiked MIBAS samples was low and several significant problems were encountered in the performance of the toxicity test protocols. We have determined that the toxicological tests specified for use in the Ocean Incineration Research Strategy are suitable for use as important assessment tools in the future. Modifications to the Mobile Bioassay Laboratory and some refinement of the test protocols are needed in order to confidently apply these protocols to at-sea research burns.

The problems encountered in validating the performance of the MIBAS sampling system must be overcome before we can confidently apply them at sea. Additional validation of the MIBAS train through additional field trials, including the use of on-site chemical determinations followed by more definitive and rigorous multicompound spiking experiments, is recommended. These additional validation runs should be performed on the research incinerator at a variety of conditions (e.g., hot furnace, fuel oil burn only; hot furnace chlorinated waste burn) followed by a repeat of the land-based toxicological testing sequence, in order to fully validate this promising methodology.

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APPENDIX A

STANDARD OPERATING PROCEDURES

TOXICOLOGICAL AND EMISSIONS SAMPLING METHODOLOGY

TABLE OF CONTENTS

Page

Preparation and Assembly of the MIBAS Sampling Train.................... A-1

1.0 Objective.. A-1
2.0 Equipment and Materials...................................... A-1

2.1 The MIBAS Description.................................... A-1
2.2 Glassware.. A-4
2.3 Reagents... A-5

3.0 Procedures... A-6

3.1 Assembly of MIBAS Train.................................. A-6
3.2 Leak Test and Final Preparation.......................... A-7

4.0 Quality Control.. A-9
5.0 Training... A-9
6.0 Safety... A-9
7.0 Attachments.. A-9

7.1 Equipment List for MIBAS: Probe......................... A-10
7.2 Equipment List for MIBAS: Probe-Sample Recovery......... A-11
7.3 Equipment List for MIBAS: Flexible Transfer Line........ A-12
7.4 Equipment List for MIBAS: Glassware-From Condenser to Fifth Impinger.. A-13
7.5 Equipment List for MIBAS: Glassware-Sample Recovery..... A-14
7.6 Equipment List for MIBAS: Pump and Gas Meter System..... A-15
7.7 Equipment List for MIBAS: Expendable Items.............. A-16

Collecting Emissions Samples Using the MIBAS Sampling Train............ A-17

1.0 Objective.. A-17
2.0 Start-up and Sampling Procedures............................. A-17

2.1 Shakedown.. A-17
2.2 Train Blank.. A-18
2.3 Collecting the MIBAS Sample.............................. A-18

3.0 Quality Control.. A-20
4.0 Training... A-20
5.0 Safety... A-20

Recovery of MIBAS Samples Prior to Water Quality Adjustment and Toxicity Testing.. A-21

1.0 Objective.. A-21
2.0 Preparation.. A-21
3.0 Procedures... A-22

TABLE OF CONTENTS
(Continued)

Page

Cleaning MIBAS Glassware Between Tests **A-23**

1.0 Objective A-23
2.0 Preparation A-23
3.0 Procedures A-23
4.0 Safety A-24

Water Quality Adjustment of Aqueous Emission Samples **A-25**

1.0 Objective A-25
2.0 Preparation A-25

  2.1 Equipment and Labware A-25
  2.2 Preparation of Labware Containers A-25
  2.3 Preparation of pH Adjusting Solutions A-26
  2.4 Special Safety Precautions A-26
  2.5 Labels and Sample Containers A-27

3.0 Procedures A-28

  3.1 Preliminary Procedures A-29
  3.2 Salinity Adjustment A-29
  3.3 pH Adjustment A-30
  3.4 Final Water Quality Adjustment A-31
  3.5 Sample Distribution A-32
  3.6 Glassware Cleaning A-32

4.0 Calculations A-32
5.0 Quality Control A-32
6.0 Training A-33
7.0 Safety A-33
8.0 Attachments A-33

  8.1 Equipment List for Water Quality Adjustments of Aqueous Samples A-34
  8.2 Example of an Emissions Sample Water Quality Adjustment Data Form A-36

Conducting Sea Urchin Sperm Toxicity Test **A-38**

1.0 Objective A-38
2.0 Test Preparation A-38

  2.1 Test Animals A-38
  2.2 Dilution Water A-38
  2.3 Test System A-39

    2.3.1 Equipment A-39
    2.3.2 Solutions A-39

TABLE OF CONTENTS
(Continued)

Page

2.3.3 Seawater Set-Ups A-39
2.3.4 Obtain Eggs and Prepare Appropriate Concentration for Test A-41
2.3.5 Obtain Sperm and Prepare Appropriate Dilution for Test A-41

3.0 Test Procedures A-42
4.0 Data Collection and Tabulation A-42
5.0 Data Analysis A-43

5.1 Test Validity A-43

6.0 Quality Control A-44
7.0 Training A-44
8.0 Safety A-45

9.0 Attachments A-45

9.1 Equipment List for Sperm Cell Toxicity Test A-46
9.2 Diagram of Recommended Setup for Conducting Serial Dilution Test A-47
9.3 Estimating Egg Concentration and Diluting to Appropriate Concentration A-48
9.4 Calibration Curve Correlating Sperm Concentration and Optical Density of Sperm Solutions A-49
9.5 Estimating Sperm Concentration and Diluting to Appropriate Concentration A-51
9.6 Sperm Cell Data Sheets A-52

Conducting Static-Renewal Rapid Chronic Toxicity Tests with *Mysidopsis bahia* **A-55**

1.0 Objective A-55
2.0 Preparation A-55

2.1 Test Animals A-55

2.1.1 Culturing of *Mysidopsis bahia* for Toxicity Testing A-55
2.1.2 Feeding and Handling A-55
2.1.3 Diseased Animals A-56
2.1.4 Acquisition of Juvenile Mysids A-56

2.2 Dilution Water A-56
2.3 Description of Test System A-57

2.3.1 Test Chambers and Aquaria A-57
2.3.2 Environmental Conditions A-57

3.0 Test Procedures A-57

3.1 Test Initiation A-57

TABLE OF CONTENTS
(Continued)

Page

4.0 Data Collection A-58

4.1 Routine Biological Monitoring A-58
4.2 Routine Test Monitoring A-59

4.2.1 Water Quality A-59

5.0 Data Reduction and Reporting A-59
6.0 Quality Control A-60
7.0 Training A-60
8.0 Safety A-60
9.0 Attachments A-61

9.1 7-Day Rapid Chronic Mysid Test Biological Data Sheet A-62
9.2 7-Day Rapid Chronic Mysid Test Water Quality Data Sheet A-63
9.3 7-Day Rapid Chronic Mysid Test Summary Data Sheet A-64

Conducting Seven-Day Growth/Survival (Static Renewal) Studies with the Inland Silverside, *Menidia beryllina* A-65

1.0 Objective A-65
2.0 Preparation A-65

2.1 Test Animals A-65

2.1.1 Source and Handling A-65
2.1.2 Obtaining Embryos and Maintaining Embryos and Larvae A-66
2.1.3 Feeding A-66
2.1.4 *Artemia* Quality Control A-67

2.2 Dilution Water A-68
2.3 Description of Test System A-68

2.3.1 Test Chambers A-68
2.3.2 Experimental Design A-69
2.3.3 Test Parameters A-69
2.3.4 Test Equipment A-70

3.0 Test Procedures A-70

3.1 Test Initiation A-70
3.2 Chamber Randomization A-71
3.3 Environmental Controls A-71

4.0 Data Collection and Test Maintenance A-72

4.1 Biological and Water Quality Monitoring A-72

5.0 Data Reduction and Analysis A-75

TABLE OF CONTENTS
(Continued)

Page

6.0 Quality Control A-75
7.0 Training A-75
8.0 Safety A-76
9.0 References A-76

10.0 Attachments A-76

10.1 Equipment List for Conducting Test A-77
10.2 Data Forms for Fish Growth/Survival Study A-78

Estimating Chronic Sexual Reproduction Effects with Champia parvula **A-82**

1.0 Objective A-82
2.0 Preparation A-82

2.1 Test Organism A-82

2.1.1 Maintenance of Stock Cultures of Chempia parvula A-84
2.1.2 Unhealthy Plants A-86

2.2 Artificial Seawater A-86

3.0 Procedure A-87

3.1 Readiness of Stock Plants A-87
3.2 Preparation of Plants for a Test A-87
3.3 Protocol A-93

4.0 Data Collection A-93
5.0 Data Analysis A-94
6.0 Quality Control A-97
7.0 Training A-97
8.0 Safety A-97
9.0 References A-97
10.0 Attachments A-98

10.1 List of Materials for Performing Toxicity Tests with Champia parvula A-99
10.2 Sample Data Sheet for Number of Cystocarps per Plant of Champia parvula A-100

Life-Cycle Toxicity Tests with Dinophilus gyrociliatus **A-101**

1.0 Objective A-101
2.0 Test Preparation A-101

2.1 Test Animals - Culture Maintenance and Feeding A-101
2.2 Animal Handling and Collection A-101
2.3 Glassware Cleaning A-102
2.4 Food Preparation A-103
2.5 Test Equipment A-103

TABLE OF CONTENTS
(Continued)

Page

3.0 Test Procedures........ A-103
- 3.1 Test Setup........ A-103
- 3.2 Test Initiation........ A-103

4.0 Data Collection........ A-104
- 4.1 Water Quality Data........ A-104
- 4.2 Biological Data........ A-104

5.0 Data Analysis........ A-105
6.0 Quality Control........ A-105
7.0 Training........ A-105
8.0 Safety........ A-106
9.0 Attachments........ A-106
- 9.1 Equipment List for _Dinophilus_ Life-Cycle Test........ A-107
- 9.2 Example of _Dinophilus_ Water Quality Monitoring........ A-108
- 9.3 Example of _Dinophilus_ _gyrociliatus_ Toxicity Monitoring Sheet.. A-109
- 9.4 Example of _Dinophilus_ _gyrociliatus_ Reproduction Data Sheet.... A-110

LIST OF FIGURES

1.0 Schematic Diagram of the Assembled MIBAS Train........ A-2
2.1 Life History of the Marine Red Alga _Champia_ _parvula_........ A-82
2.2 Apical Tip of Female Branch of _Champia_ _parvula_ Showing Sterile Hairs and Trichogynes (Reproductive Hairs)........ A-88
2.3 A Portion of a Male Thallus of _Champia_ _parvula_ Showing Spermatial Sori........ A-89
2.4 A Close-up of a Portion of a Spermatial Sorus........ A-90
2.5 Apical Tip of a Female of _Champia_ _parvula_ That Had Been "Dipped" in Water From a Male Culture........ A-91
2.6 The Size and Degree of Branching that is Generally Used for the Female Starting Plants........ A-92
2.7 A Mature Cystocarp of _Champia_ _parvula_........ A-95
2.8 Comparison of a Very Young Branch with an Immature Cystocarp....... A-96

LIST OF TABLES

1.0 Recipe for Additional Nutrients to be Added to Natural Seawater for _Champia_ _parvula_ Stock Cultures and Test Medium........ A-85

**U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF MARINE AND ESTUARINE PROTECTION
INCINERATION-AT-SEA RESEARCH PROGRAM**

STANDARD OPERATING PROCEDURES

FOR

PREPARATION AND ASSEMBLY OF THE MIBAS SAMPLING TRAIN

1.0 OBJECTIVE

The Marine Incineration Biological Assessment Sampler (MIBAS) train is designed to collect emissions from at-sea incinerator systems for aquatic bioassay tests. These Standard Operating Procedures (SOP) provide directions for the on-site preparation and assembly of the MIBAS sampling train prior to initiation of sampling. Procedures for initial laboratory preparation, operation, and shipping the MIBAS train to the test site are provided in companion SOPs.

2.0 EQUIPMENT AND MATERIALS

2.1 The MIBAS Description.

2.1.1 The MIBAS sampling train is illustrated in Figure 1.

2.1.2 The MIBAS probe is constructed of double stainless steel jacketing enclosing a quartz probe liner. The quartz sampling line is 1.3-cm (1/2-in) OD. The outer stainless steel jacket contains cooling water, and the inner sleeve contains cooling air. Probe lengths are 1.8-m (6-ft) and 7.3-m (24-ft). The shorter length probes are used for land-based tests, and the longer probes are used for sea studies aboard incineration ships.

2.1.3 A Teflon transfer line is used to connect the sampling probe to the MIBAS cooling condenser. This line is 1.5-m (5-ft) in length and is jacketed with heat tape. The heat tape is wrapped with

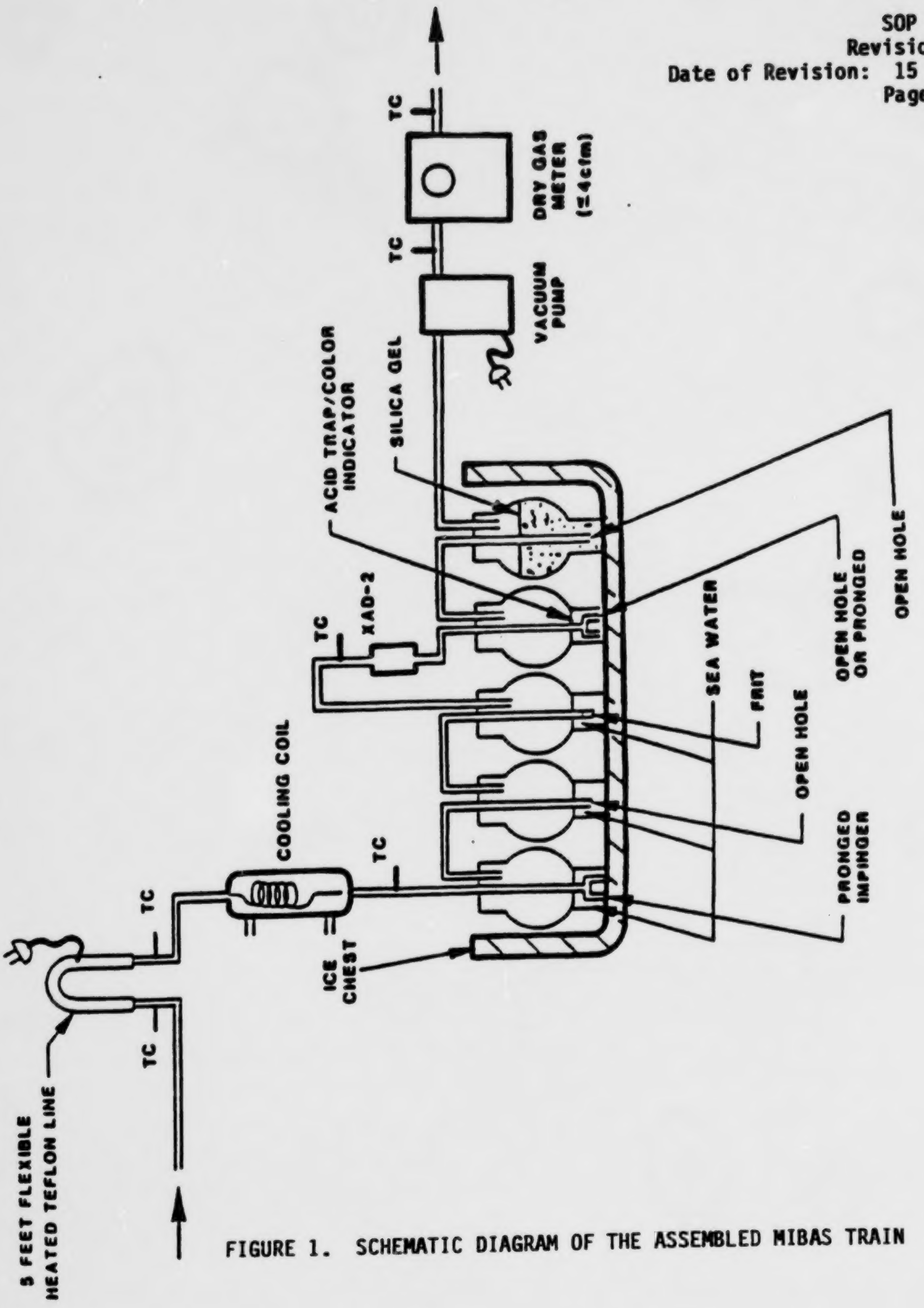
5 FEET FLEXIBLE HEATED TEFLON LINE
TC
TC
TC
COOLING COIL
TC
ICE CHEST
PRONGED IMPINGER
OPEN HOLE
FRIT
SEA WATER
TC
XAD-2
OPEN HOLE OR PRONGED
OPEN HOLE
ACID TRAP/COLOR INDICATOR
SILICA GEL
VACUUM PUMP
TC
DRY GAS METER (≤4cfm)
TC

FIGURE 1. SCHEMATIC DIAGRAM OF THE ASSEMBLED MIBAS TRAIN

insulation and is covered with a protective plastic shield.

2.1.4 All impingers and transfer lines in the MIBAS train are connected with 28/15 standard ball-socket joints. Thermocouple wells are fabricated from 10-cm (3/8-in) OD threaded-well, glass joints. The thermocouple is held against a Viton O-ring with a threaded, male Nylon bushing. The Viton O-ring is carefully wrapped with Teflon tape before each sampling episode to prevent organic contamination.

2.1.5 Thermocouples are 3-cm (1/8-in) OD made of chromel-alumel shielded with stainless steel. Temperature monitoring points are indicated in Figure 1. Critical temperatures are the probe temperature, temperature at the inlet of the first impinger and the temperature of the gas stream at the dry gas meter.

2.1.6 The gas stream is cooled with a jacked, cold water condenser placed before the first impinger. The condenser has outside dimensions of 10-cm (4-in) OD by 30-cm (12-in) length. A submersible liquid pump is placed in a two-phase ice chest (ice-water) and is used to cycle cold water through the condenser. Gas entering the first impinger should be below 22°C (74°F) for proper operation of the MIBAS train.

2.1.7 Three impingers are used in series to collect the MIBAS sample. The impingers have a liquid capacity of 21 and are made in two pieces held together with tapered glass fittings. The impingers can be dismantled for cleaning.

The first impinger has a four-pronged outlet extending from the impinger stem. The openings are nominally 0.5-cm (3/16-in) OD which allows flue gas particulate to pass into the first impinger without plugging the train.

The second impinger has a straight, open end stem. The second impinger acts as an overflow chamber in case excess moisture is condensed in the first impinger.

The third impinger contains a flutted impinger stem which opens to a 3-cm (1-1/4-in) OD funneled opening that contains a fritted glass disk (Grade A). The high porosity frit creates small bubbles and improves gas-liquid contact.

2.1.8 An XAD-2 cartridge is placed in line after the third impinger. This cartridge contains 120 g of pre-cleaned XAD-2 resin. The two ends of the resin chamber contain glass frits to allow gas passage while supporting the resin bed. The XAD-2 resin is cleaned and blank checked using the procedures described in the EPA Level 1 Assessment Manual (IERL, 1978).

2.1.9 The fourth impinger protects the train. 500 ml of caustic solution (0.2 N NaOH) containing bromcresol purple indicator is placed in the last (fourth) impinger. The purpose of the caustic solution is to trap any HCl that passes through the train. The indicator solution can be observed by the operator to show HCl breakthrough. If a color change occurs in the base solution, the sampling episode should be halted at once since damage to the pump and dry gas meter can occur if HCl vapor is allowed to contact metal surfaces.

2.2 Glassware

2.2.1 1-l and 500-ml volumetric flasks.

2.2.2 Miscellaneous beakers and flasks for rinsing glassware and MIBAS train.

2.2.3 Storage containers are 1-l and 1-gal glass bottles.

2.2.4 All storage bottles containing standards or samples shall be light protected, either by use of amber glass bottles, or covered with aluminum foil, or stored in the dark. Stored samples or standards will be held at 4°C either in an ice chest or refrigerator until the time of analysis.

2.2.5 Cleaning procedure for glassware.

2.2.5.1 Wash with laboratory detergent.

2.2.5.2 Rinse with tap water.

2.2.5.3 Rinse with distilled, deionized water (DDI) water.

2.2.5.4 Rinse with distilled-in-glass (DIG) or equivalent grade methanol (MeOH).

2.2.5.5 Rinse with DIG methylene chloride (MeCl).

2.2.5.6 Rinse with DIG MeOH (3X).

2.2.5.7 Rinse with DDI water (3X).

2.2.5.8 Anneal overnight (16 hours minimum) in a muffle or other suitable, contamination free oven at 400°C (752°F).

2.2.5.9 Hold all glassware under closure, either by capping with clean aluminum foil, or cap. Clean aluminum foil with sequential rinses: 1. MeCl rinse, 2. MeOH rinse, 3. DDI rinse.

2.2.5.10 All caps must be lined with Teflon disks.

2.2.6 (Exceptions) Cleaning procedures may be modified in field locations (e.g., shipboard). Where DDI water is not available, step 2.2.5.3 is ammended to allow distilled (DW) water, and where a suitable oven is not available for annealing, step 2.2.5.8 can be ammended to allow air drying; however, air drying should be performed in a contamination-free and insect-free environment.

2.3 Reagents

2.3.1 Distilled, deionized water.

2.3.2 Indicating silica gel, 6-16 mesh. Prepare by heating overnight (minimum 16 hours) in a 104°C oven.

2.3.3 Seawater - Ocean water shall be heated to 80°C and filtered with a 0.5-µm particulate filter. Seawater is stored and transported in linear polyethylene or Teflon containers.

2.3.4 0.20 N NaOH - To prepare the caustic solution for the backup impinger, dissolve 8.0 g of NaOH pellets in 200 mL of distilled water in a 1-l volumetric flask and cool to room temperature. Fill to the 1-l mark with distilled water.

2.3.5 Methanol, methylene chloride or any other organic solvents used should be "distilled-in-glass" or equivalent purity.

2.4 Miscellaneous Reagents and Supplies

2.4.1 Aluminum foil, cleaned as given above in 2.2.5.9.

2.4.2 Ice/water.

2.4.3 Balance - analytical, capable of accurately weighing 0.0002 g.

2.4.4 Teflon wash bottles, as needed, clearly labelled.

3.0 PROCEDURES

3.1 Assembly of MIBAS Train

3.1.1 Label a laboratory notebook or data sheet with the date, location, test number, project number, and any other information pertinent to the current MIBAS experiments. Remove all MIBAS components from shipping containers.

3.1.2 Rinse impingers, Teflon lines, cross-over tubes and probe with methanol, and allow to completely air dry.

3.1.3 Place the impinger support rack in an ice chest and mount the five impingers in the rack. Fill the impingers as follows:

Impinger 1	500 ml seawater
Impinger 2	500 ml seawater
Impinger 3	500 ml seawater
Impinger 4	500 ml 0.40 N NaOH
Impinger 5	700 g indicating silica gel

3.1.4 Attach the cross-over tubes in consecutive order from outlet of Impinger 1 through Impinger 3 using ball-joint clamps.

3.1.5 Record the XAD-2 cartridge code in a laboratory notebook. Insert the XAD-2 column between the outlet of Impinger 3 and the inlet of Impinger 4 as shown in Figure 1. Cover the XAD-2 column with aluminum foil to keep out light.

3.1.6 Attach a cross-over tube from the outlet of Impinger 4 to the inlet of Impinger 5.

3.1.7 Connect the outlet of Impinger 5 to the copper line leading to the dry gas meter. Place 7.5-9 m (25-30 ft) of copper line between the vacuum pump exhaust and the dry gas inlet. This tubing is necessary to condition the gas before entering the dry gas meter.

3.1.8 Attach the thermocouple wells and the condenser to the train as shown in Figure 1.

3.1.9 Attach the 1.5-m (5-ft) flexible Teflon sample line to the condenser inlet and cap the inlet of the sample line with a ball cap. Loosen clamps on all ball joints.

3.2 Leak Test and Final Preparation

3.2.1 Uniformly tighten all the ball joint clamps to a constant pressure, finger tight. Verify that the by-pass valve of the vacuum pump is closed then turn on the vacuum pump and allow it to stabilize at 15 in Hg or less vacuum. Slowly open the control valve to the full open position. Stop quickly if no response is observed to partial vacuum or if a loud whistling is heard. When a vacuum gauge response is observed, open to the full open position. Avoid prolonged operation when leaks are observed since this can lead to contamination of the train.

3.2.2 Once the vacuum pump is stabilized, the dry gas meter should read <0.02 cfm. If the reading is >0.02 cfm, close the control valve, turn off the vacuum pump, and proceed to steps 3.2.2.1 through 3.2.2.5. If the dry gas meter reads <0.02 cfm, proceed to Step 3.2.3.

3.2.2.1 Examine all ball joints for proper alignment and check for tightness. Correct as necessary. Turn on the vacuum pump, slowly open the control valve, and check the dry gas meter. If the reading is <0.02, proceed to Step 3.2.3.

3.2.2.2 If the dry gas meter still reads >0.02, apply distilled, deionized water to all ball joints with a water bottle. If bubbling is detected at any joint, turn off the control valve at the inlet to the vacuum pump (but not the vacuum pump itself) and slowly remove the end cap from the sample line inlet to equalize system the sample pressure.

3.2.2.3 Wipe any joint at which bubbling was detected with a tissue to remove any foreign matter.

3.2.2.4 Reassemble the joints. Replace any joint that still leaks with a new joint. Continue checking joints until the dry gas meter reads <0.02 cfm.

3.2.3 When the MIBAS train stabilizes at <0.02 cfm, record a final dry gas meter reading in the laboratory notebook, and turn off the valve at the vacuum pump inlet.

3.2.4 The complete leak check is performed with the sample probe installed. Close the main control valve and slowly release the system vacuum by removing the cap from the Teflon transfer line. Attach the sample probe. The sample end of the probe is capped with heavy wall rubber tubing that has a critical orifice in line. On the end of the rubber tubing a hose clamp is attached to seal the probe and release vacuum. The critical orifice prevents an accidental surge if vacuum is released.

3.2.5 Repeat the leak check with the probe installed.

4.0 QUALITY CONTROL

A leak check should be performed before, and after, each sampling episode. The QC officer present should initial the vacuum readings for the successful pre- and post-test leak checks in the laboratory notebook.

5.0 TRAINING

All operators using the MIBAS train should have instruction on the proper operation of the train. This instruction shall be accomplished by assembling the train and achieving a suitable leak check in the presence of a trained MIBAS operator. The training shall be recorded and the trained observer shall initial the notebook for the day that this training occured.

6.0 SAFETY

Operations that involve solvents such as methylene chloride (a suspect carcinogen) should be conducted in a fume hood approved by the laboratory safety officer. In addition, all operators should use impermeable plastic gloves when handling the glassware, especially during cleaning operations that involve organic solvents. Hand protection is also required when handling hot glassware. Safety glasses will be used at all times. No food or beverages will be allowed in active laboratories or in field locations where the MIBAS train is operated.

7.0 ATTACHMENTS

Attachment 1.	Equipment List for **MIBAS Train: Probe**
Attachment 2.	Equipment List for **MIBAS Train: Probe-Sample Recovery**
Attachment 3.	Equipment List for **MIBAS Train: Flexible Transfer Line**
Attachment 4.	Equipment List for **MIBAS Train: Glassware-From Condenser to** Fifth Impiger
Attachment 5.	Equipment List for **MIBAS Train: Glassware-Sample Recovery**
Attachment 6.	Equipment List for **MIBAS Train: Pump and Gas Meter System**
Attachment 7.	Equipment List for **MIBAS Train: Expendable Items**

ATTACHMENT 1. EQUIPMENT LIST

FOR MIBAS TRAIN

Probe

Quantity	Item	Check
_______ ea.	Probe, Protective Outer Jacket, Water-Cooled 24 ft	_____
_______ ea.	Quartz Probe Liner, 24 ft, Wrapped and Tested	_____
_______ ea.	Orifice Assembly - for Leak Check	_____
_______ ea.	Gland Packing, Palfoil Graphite Tape #1401	_____
_______ ea.	Clamp, #28 for Ball and Socket Joint	_____
_______ ea.	Variac, 10 amp - 110V for Probe Heater	_____
_______ ea.	Water Hose, Garden 3/4" - 100 ft	_____
_______ ea.	Hose Clamps, Assortment of Sizes, 1/2", 1", 2", 3"	_____
_______ ea.	Wrenches, Adjustable	_____
_______ ea.	Screwdrivers, 1/4" Blade	_____

ATTACHMENT 2. EQUIPMENT LIST

FOR MIBAS TRAIN

Probe - Sample Recovery

Quantity		Item	Check
	ea.	Brush, Nylon Bristle - Threaded End	
	ea.	Attachment for Probe Brush - Teflon Tubing, 1/4" O.D. x 25'	
	ea.	Amber Sample Bottle, 1 gal (Saftepak)	
	ea.	Amber Sample Bottle, 1 qt - N/M - for Solvent Rinse	
	ea.	Wash Bottles, Teflon Squeeze, 1 qt	
	ea.	Labels, Pre-printed on Poly Paper	
	ea.	Labels, Metal 1" x 2" with Wire	
	ea.	Solvents, Methyl Alcohol + Methylene Chloride	
	ea.	Solvent, Methylene Chloride (Saftepak)	
	ea.	Solvent, Distilled Water (Saftepak)	
	ea.	Tape, Mylar 3" wide x 50 yd	
	ea.	Plastic Bags, to Fit 1 gal Jugs	
	ea.	Chain-of-Custody Forms	
	ea.	Data Sheets	

ATTACHMENT 3. EQUIPMENT LIST

FOR MIBAS TRAIN

Flexible Transfer Line

Quantity	Item	Check
______ ea.	Heated, Teflon Line, 5 ft MFG #212-10-5	______
______ ea.	Variac - 5 amp - 110V	______
______ ea.	Ball Joint Fitting - 28/12 Male to 1/2" Tube	______
______ ea.	Ball Joint Fitting - 28/12 Female to 1/2" Tube	______
______ ea.	Ball Joint Clamps - #28	______
______ ea.	Hose Clamps, 1/2" - 3/4"	______
	FOR SAMPLE RECOVERY	
______ ea.	Nylon Brush, Threaded	______
______ ea.	Tubing, 6', S.S. Threaded - inside	______
	The other items are the same as used on probe sample recovery	

ATTACHMENT 4. EQUIPMENT LIST

FOR MIBAS TRAIN

Glassware - From Condenser to Fifth Impinger

Quantity	Item	Check
________ ea.	Condenser, Cooling - 6" Dia. x 12" with Quick Disconnect Fittings	____
________ ea.	Pump, Water Circulating - Little Giant Pump Co. Part #1P-372, Model 2E-38N, with 3/8" x 1/4P Hose Bibs	____
________ ea.	Tygon Tubing - 3/8"	____
________ ea.	Quick Disconnects, 3/8" Poly Propylene	____
________ ea.	"T" Fitting for Thermcouple - 3"	____
________ ea.	First Impinger Bottle, 2.5 liter with 45/50 ₮	____
________ ea.	First Impinger Stopper - 45/50 ₮ with 4-Prong Opening	____
________ ea.	Cross-over U-Tube Connector	____
________ ea.	Second Impinger Bottle, 2.5 liter, with 45/50 ₮	____
________ ea.	Second Impinger Stopper - 45/50 ₮ Open-end	____
________ ea.	Cross-over U-Tube Connector	____
________ ea.	Third Impinger Bottle, 2.5 liter with 45/50 ₮	____
________ ea.	Third Impinger Stopper, 45/50 ₮ Fritted-end	____
________ ea.	Connector, Straight - 12"	____
________ ea.	Cross-over U-Tube Connector with TC Well	____
________ ea.	XAD-2 Cartridge - 200 gram Capacity	____
________ ea.	Fourth Impinger Bottle, 2.5 liter with 45/50 ₮	____
________ ea.	Fourth Impinger Stopper, 45/50 ₮ with 4-Prong Opening	____
________ ea.	NaOH Pellets, - 6 grams Sealed in a P.E. Tubing	____
________ ea.	Fifth Impinger Bottle, 2.5 liter with 45/50 ₮	____
________ ea.	Fifth Impinger Stopper, 45/50 ₮ Open-end	____
________ ea.	Silca Gel, 700 gram Packed in Zip Lock Bag	____
________ ea.	Can, Friction Top, 1/2 gal for Silica Gel	____
________ ea.	Glass Fitting, Elbow 28/12 x 1/2" Tube	____
________ ea.	Clamps, #28 for Ball and Socket Joint	____

ATTACHMENT 5. EQUIPMENT LIST

FOR MIBAS TRAIN

Glassware - Sample Recovery

Quantity	Item	Check
________ ea.	Same as described for Probe-Sample Recovery - Impinger Catches and Water Rinses are Combined into a 1 gal Container	______
	ADDITIONAL ITEMS	
________ ea.	Measuring Cylinder, 500 mL, Poly Propylene	______
________ ea.	Measuring Cylinder, 250 mL, Poly Propylene	______
________ ea.	Measuring Cylinder, 100 mL, Poly Propylene	______
________ ea.	Funnel, 6", Poly Propylene	______
________ ea.	Sample Data Sheets	______
________ ea.	Teflon Wash Bottles	______

ATTACHMENT 6. EQUIPMENT LIST

FOR MIBAS TRAIN

Pump and Gas Meter System

Quantity	Item	Check
________ ea.	Vacuum Pump, 4 cfm - Doerr Model #D272X 3/4 HP, 115V, 11 amp	______
________ ea.	Vacuum Hose, Red Rubber, 1/2" I.D.	______
________ ea.	Copper Tubing, for Cooling Pump Exhaust - 1/2" I.D. x 25 ft	______
________ ea.	Dry Gas Meter, Rockwell Model 3/4" - R200 with Thermocouples at Inlet and Outlet	______
________ ea.	Digital Readout	______
	OTHER ITEMS	
________ ea.	Ice Chest - 72 qt Capacity, Fitted with Customized Impinger Hold-downs	______
________ ea.	Stop Watch	______
________ ea.	Internal Timer with Alarm	______
________ ea.	Clip Board, Aluminum Case, Snapak, Sanders Mfg. Co., Winthrope, Massachusetts	______
________ ea.	Data Sheets - Printed on Water-Proof Paper	______
________ ea.	Barometer	______
________ ea.	Plastic Buckets, 5 gal	______
________ ea.	Extension Cords	______
________ ea.	Face Mask	______
________ ea.	Foundryman's Jacket - Aluminized Fabric	______
________ ea.	Gloves, Kevlar 29	______
________ ea.	Tool Box	______
________ ea.	Measuring Tape	______

ATTACHMENT 7. EQUIPMENT LIST

FOR MIBAS TRAIN

Expendable Items

Quantity		Item	Check
________	ea.	Aluminum Foil	____
________	ea.	Towels	____
________	ea.	Tissues, Wipes	____
________	ea.	Tape, Electrical - Glass	____
________	ea.	Tape, Electrical - #33	____
________	ea.	Tape, Duct	____
________	ea.	Pens	____
________	ea.	Universal pH Indicator Paper	____
________	ea.	Polyethylene Sheeting	____
________	ea.	Tablets	____
________	ea.	First Aid Kit	____
________	ea.	Calendar	____

SOP No. 3-02
Revision No. 01
Date of Revision: 15 May 1986
Page 1 of 4

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF MARINE AND ESTUARINE PROTECTION
INCINERATION-AT-SEA RESEARCH PROGRAM

STANDARD OPERATING PROCEDURES

FOR

COLLECTING EMISSIONS SAMPLES USING THE MIBAS SAMPLING TRAIN

1.0 OBJECTIVE

This SOP provides directions for initiating and acquiring an emission sample using the MIBAS sampling train. It is assumed that all procedures required in the preparation and assembly SOP have been completed.

2.0 START-UP AND SAMPLING PROCEDURES

2.1 Shakedown

The purpose of the shakedown is to establish the control points to use during actual sampling. Mount the train and turn on the cooling water and air. Start the condenser cooling water. Lift the probe into place for insertion into the stack. Start the sampling pump and open the pump by-pass valve. Observe the pump pressure reading and the dry gas meter flow rate. Adjust the pump bypass valve to produce 2.0 cfm ± 10 percent. Record the pressure that corresponds to this flow. Verify that all thermocouples are operating properly.

Insert the sample probe in the stack to full insertion. Allow equilibration, and set the probe cooling air and water to the necessary rates that produce 120-150°C (250-350°F) at the outlet of the Teflon transfer line. (The Teflon transfer line inlet is a good temperature to monitor since the attainment of the proper temperature range here generally takes long enough to allow other temperatures to equilibrate). Visually monitor temperatures using a multi-channel digital readout meter. (Attaching the thermocouples to a variable time sequencing device is useful since manually switching thermocouples is eliminated.) Adjust the flow rate in the system to approximately 2.0 cfm by adjusting the inlet valve and/or the by-pass valve on the pump. Note the pressure drop on the pump vacuum gauge. If the desired flow can be obtained by adjusting the by-pass valve with the inlet valve in the open position, after the system has stabilized, shut off only the inlet valve. Do not change the by-pass valve.

Record final control settings (1. Approximate flow rate of cooling water and air, or note the control valve settings. 2. Variac settings for auxilliary heating, e.g., Teflon line heater. 3. Pressure gauge reading. 4. Gas meter flow rate. 5. Temperature readings.)

2.2 Train Blank

The train blank is collected by holding the train for the designated sampling period without collecting flue gas. First mount the train including the probe, at the sampling site, preferably in the locale where actual samples will be collected. It is not necessary to connect the pump or dry gas meter. Cap the ends of the train and time three-hours. Do not heat or cool the train. At the end of the three hour period, demount the train and recover the train blank sample. The sample recovery procedure (SOP For Recovery of MIBAS Samples Prior to Water Quality Adjustment) should be used. This sample should be coded and analyzed to show background contamination from handling, shipping, and storage.

2.3 Collecting The MIBAS Sample

Mount the train and perform the leak checks described in SOP for Preparation and Assembly of the MIBAS Sampling Train. Turn on all the regulated systems: cooling air and water for the probe, condenser cooling water, and Teflon line heater if it is needed. Refer to the shakedown data and set the control valves at the approximate setting needed to achieve 2.0 cfm flow. Insert the probe into the stack. Start the sampling pump. Check the vacuum reading and measure the flow rate. If the gas-phase injector system is used, start the injection simultaneously with the gas meter reading. Record the initial volume of injection solution. Re-adjust the control valve as necessary to achieve 2.0 cfm. Continue sampling for three hours.

Monitor all temperatures (probe inlet, probe outlet, transfer line outlet, MIBAS inlet, XAD-2 cartridge inlet, dry gas meter inlet, and dry gas meter outlet), and record at 15-minute intervals in the laboratory notebook. Be sure to maintain the inlet temperature into the first impinger and into the XAD-2 column below 22°C (70°F) using the condenser cooling rate. These temperatures generally run between 7° and 12°C (45-55°F).

Record the following data at 15-minute intervals:

1) Clock time and elapsed time.

2) Temperature at all locations.

3) Dry gas meter reading.

4) Volume of injected sample (if injector is used).

5) Pressure drop as read on the pump gauge.

6) Any other data pertinent to the run.

Add ice to the insulated ice chest (after draining excess H_2O) as needed. Be sure ice is packed around impinger bottoms.

After 3 hours, turn off the vacuum pump inlet valve, and turn off the submersible water pump. Allow the system pressure to equalize. Cap the probe at the inlet to the Teflon sample line with a glass ball joint and clamp. Leak check the train (See SOP for Preparation and Assembly of the MIBAS Sampling Train). After the leak check, remove the cap from the sample line inlet slowly to equalize pressure. Disconnect the vacuum pump and cap the train inlet and outlet with glass caps. Remove the Teflon sample line, cap, and set aside to cool. Remove and cap the condenser. Drain water from the ice chest. Remove the XAD-2 column and cap with glass balls and store in ice. Turn off the cooling water and air supply to the sample probe.

3.0 QUALITY CONTROL

The collected sample volume must total 10 m^3 (360-ft^3) ± 10 percent. The temperature of the XAD-2 must be kept at <22°C (<70°F). The Teflon sample line temperature must be maintained at >250°F and >400°F. Temperatures at all thermocouple locations should be taken every 15 minutes to assure the train is operating within the required temperature ranges.

4.0 TRAINING

The operator should be trained by conducting the sample shakedown (Step 2.1) under the supervision of an experienced operator. Successful completetion of the shakedown shall be documented.

5.0 SAFETY

At any field location, staff members are required to wear safety glasses, ear protection, a hard hat, and steel toed safety shoes, while at the sampling site. No food or beverages shall be allowed in the sampling area.

SOP No. 3-03
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Page 1 of 2

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF MARINE AND ESTUARINE PROTECTION
INCINERATION-AT-SEA RESEARCH PROGRAM

STANDARD OPERATING PROCEDURES

FOR

RECOVERY OF MIBAS SAMPLES PRIOR TO WATER QUALITY ADJUSTMENT AND TOXICITY TESTING

1.0 OBJECTIVE

The purpose of this SOP is to describe recovery MIBAS emission samples by brushing the probe while rinsing with natural seawater (or 30 parts per thousand brine made from concentrated seawater), thoroughly rinsing all other glassware with seawater (or brine) to recover all liquid and particulate matter into a combined liquid sample of <4 liters. This sample will subsequently be adjusted for pH and salinity prior to toxicity testing.

2.0 PREPARATION

2.1 If natural seawater is not available, prepare brine according to the procedure for preparing 30 parts per thousand brine.

2.2 Prepare sample bottles by rinsing three times with deionized (DI) water and follow with one rinse of seawater.

2.3 Assemble the probe cleaning rod sections with a Nylon bristle test, tube brush attached to the rod end.

2.4 Prepare a large funnel for collecting rinses. Clean 1-l and 4-l graduated cylinders as in 2.2.

3.0 PROCEDURES

3.1 The sample probe and heated Teflon transfer line (umbilical) are rinsed for particulate recovery at the sampling site. The sample probe is attached to a clearly labelled collection flask and brushed three times. During brushing seawater is administered to the top of the inclined probe. The probe is rotated, and brushed, while rinses are collected in the receiving flask. The brushing procedure is repeated three times. Contents of the collection bottle are immediately visually checked to verify that no solvents are present, then the sample is transferred with rinsing into a polyethylene screw cap bottle. This procedure is then repeated for the flexible sample line.

3.2 Contents of Impingers 1, 2, and 3 are individually measured in a graduated cylinder. The individual volumes are recorded and added to a 1-gallon polyethylene, screw cap bottle. Impinger flasks are rinsed three times with seawater. Impinger stems are brushed and rinsed three times with seawater. All rinses are added to the 1-gal bottle.

3.3 The contents of Impinger 4 are measured, the volume is recorded, then the liquid is discarded. The impinger and its stem are rinsed with DI water which is discarded. The solvent cleaning procedure is not used.

3.4 The stem of Impinger 5 is removed. The outside of the impinger flask is then dried and the flask plus silica gel, is weighed to 0.1 g. Record this weight. The silica gel is then discarded in normal waste. The impinger is not solvent cleaned.

3.5 All glass tubing and connections in the train, through Impinger 3, are brushed and rinsed three times with seawater. Add these rinses to the 1-gal sample bottle.

3.6 The rinses from the probe and Teflon transfer line (3.1) are added to the combined samples from 3.2 and 3.5 above.

3.7 The total sample is submitted for water quality adjustment and toxicity testing. All glassware is cleaned for further use.

SOP No. 3-04
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Page 1 of 2

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF MARINE AND ESTUARINE PROTECTION
INCINERATION-AT-SEA RESEARCH PROGRAM

STANDARD OPERATING PROCEDURES

FOR

CLEANING MIBAS GLASSWARE BETWEEN TESTS

1.0 OBJECTIVE

The objective of glassware cleaning procedures are to remove surface contamination from the MIBAS train glassware without using potentially toxic cleaning media (i.e., chromic acid), or employing techniques unavailable on shipboard laboratories.

2.0 PREPARATION

2.1 Fill labelled Teflon wash bottles with "distilled-in-glass" solvents.

1. MeCl
2. MeOH

2.2 Provide waste solvent receptacles.

3.0 PROCEDURES

3.1 After the sample has been recovered, all glassware is throughly cleaned using procedures 2.2.5.1 through 2.2.5.9 in the SOP for Preparation and Assembly of the MIBAS Sampling Train. The glassware may then be air-dried, and immediately put into service.

3.2 Care must be taken to reach all surfaces of the condenser coil. This is accomplished by turning the condenser during rinsing.

3.3 The sample probe and flexible sample line are cleaned with brushing during the rinse sequences described in 3.1.

4.0 SAFETY

Operations that involve solvents such as methylene chloride (a suspected carcinogen) should be conducted in a fume hood. In addition all operators should use impermeable plastic gloves when handling the glassware, especially during cleaning operations that involve organic solvents. Hand protection is also required when handling hot glassware. Safety glasses will be used at all times. No food or beverages will be allowed in active laboratories or in field locations where the MIBAS train is operated.

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF MARINE AND ESTUARINE PROTECTION
INCINERATION-AT-SEA RESEARCH PROGRAM

STANDARD OPERATING PROCEDURES

FOR

WATER QUALITY ADJUSTMENT OF AQUEOUS EMISSION SAMPLES

1.0 OBJECTIVE

This procedure describes the adjustment of water quality parameters of aqueous emission samples (MIBAS samples) generated during land-based trial incineration burns and at-sea research burns of hazardous wastes. This procedure has been developed to allow toxicity tests to be conducted with MIBAS samples.

A stock solution of a water quality-adjusted sample will be prepared to provide an aqueous medium suitable for marine toxicity testing. Specifically, salinity will be adjusted to $30^{o}/oo \pm 1^{o}/oo$ and pH will be adjusted to 8.0 ± 0.1. The water quality-adjusted emissions sample will consist of 10-percent original, unadjusted sample (including seawater rinses of the sampling probe and impingers) and 90-percent water quality-adjusting medium and seawater.

2.0 PREPARATION

2.1 Equipment and Labware

The supplies and equipment needed to perform this procedure are listed in Attachment 1. Plastics (i.e., polyethylene or polycarbonate) or Teflon-lined plastics are used wherever possible rather than glass, due to the high potential for breakage of glassware products at sea.

2.2 Preparation of Labware Containers

The following procedure will be followed to clean all new or reused labware. No solvents will be used for cleaning of sample containers or any containers used during the water quality adjustment procedure.

1. Rinse three times with deionized water.
2. Air-dry on drying rack and store in dust-free environment.
3. Rinse once with 30 $^{o}/oo$ seawater just prior to use.
4. Between cruises, provide a detergent wash, rinse, acid bath, and rinse procedure.

2.3 Preparation of pH Adjusting Solutions

Four pH adjusting solutions will be prepared as follows:

1. **10N NaOH**. Dissolve 200 g NaOH pellets in 500 ml deionized water.
2. **1N NaOH**. Dilute 10N NaOH solution, 1 part 10N NaOH:9 parts deionized water.
3. **0.1N NaOH**. Dilute 1N NaOH solution, 1 part 1N NaOH:9 parts deionized water.
4. **10N HCl**. Add 85 ml deionized water to 500 ml volumetric flask, then bring to 500 ml volume with concentrated HCl.

2.4 Special Safety Precautions

Take appropriate precautions in handling pH adjusting solutions and the test solutions. Wear protective glasses, a lab coat, and gloves when working with these caustic or potentially hazardous solutions.

2.5 Labels and Sample Containers

All labware used for water quality adjustments should be color-coded to minimize the potential for the contamination of clean labware with labware designated for unadjusted or adjusted test samples. Labware exposed to the unadjusted emission samples should be coded with red tape and labware exposed to the water quality adjustment emission samples should be coded with pink tape.

Two separate labels will be used to distinguish the unadjusted and the water quality adjusted aqueous emission samples.

a. The Unadjusted Sample consists of the combined liquids and seawater rinses from the sampling probe and the impingers. The unadjusted sample is brought to a 4-liter volume with seawater (30 ^{o}/oo). See "Procedure for Recovery of MIBAS Samples Prior to Water Quality Adjustment and Bioassay."

Samples Prior to Water Quality Adjustment and Bioassay." The Unadjusted Emissionn Sample will be labeled as:

Label 1

DI Rinsed (3x)________ Project No.____________

SW Rinsed (1x)________ Date__________ Time__________

UNADJUSTED EMISSION SAMPLE

Designation:__________________________________

Impinger (3) Liquid + Rinses Added____________

Probe Rinse Added______________Final Volume_________

Collected By____________Transf. To________________

Purpose_____________________Initials_______________

b. The Water Quality Adjusted Sample will be prepared from a 200-ml subsample of the unadjusted emission sample and will be labeled as:

Label 2

DI Rinsed (3x)_______ Project No.___________

SW Rinsed (1x)_______Date _______Time ____________

EMISSION SAMPLE
WATER QUALITY ADJUSTED

Sample Designation:______________________________

Volume_________Salinity_________pH________________

Prepared By__________Transf. To__________________

Purpose_____________________Initials_____________

3.0 PROCEDURES

Initially these procedures were developed for two types of samples, acid-spiked samples which simulate the anticipated characteristics of samples generated during hazardous waste burns and non-acid-spiked, fuel oil emission samples. Seven MIBAS samples generated during land-based trials in March 1986 were also adjusted according to the following procedures. The procedure outlined below has been generalized so that it will be adaptable to emission samples of varying character. The unadjusted emission samples are delivered for water quality adjustment in a tightly capped, 1-gallon jug. Specific volumes of pH adjusting media, deionized water, concentrated brine solution, or 30 $^{0}/oo$ seawater may vary with each sample.

It is important to measure the initial salinity and pH and to make frequent checks as the water quality adjustment proceeds. A subsample of the emission samples will be adjusted to a final salinity of 30 $^{0}/oo$ ($\pm$ 1 $^{0}/oo$) and a pH of 8.0 ($\pm$ 0.1 $^{0}/oo$) and then diluted with seawater to a final strength of 10-percent MIBAS.

3.1 Preliminary Procedures

1. Shake the 1-gallon jug containing the unadjusted sample by inverting five times.
2. Uncap and pour off a measured subsample into a graduated cylinder.
3. Transfer the subsample to a beaker and stir on a magnetic stir plate.
4. Measure and record salinity and pH on the Water Quality Adjustment Data Form (Attachment 2).
5. Transfer the subsample to a container of sufficient size to accommodate mixing and a 10-fold volume expansion. Place on magnetic stir plate and stir.

   (E.g., for a 1000 ml subsample, transfer to a 1000 ml beaker for salinity and pH measurement. Then transfer the subsample to a 20-liter carboy on a stir plate.)

3.2 Salinity Adjustment

3.2.1 Samples of Salinity Greater Than 30 ^{o}/oo

1. Add an appropriate volume of deionized water to adjust the salinity to approximately 26 ^{o}/oo. Check salinity with a refractometer.

   The volume of deionized water required to dilute the MIBAS sample to 26 ^{o}/oo is determined by the following calculations:

a) $$\frac{\text{Target Salinity (26}^{o}\text{/oo)}}{\text{Initial Salinity}} \times 1000 \text{ ml} = \frac{\text{Proportional Vol. (ml) of}}{\text{MIBAS Required Per Liter}}$$

b) $$1000 \text{ ml} - \text{Proportional Vol. MIBAS} = \begin{array}{l}\text{Proportional Vol. Deionized} \\ \text{Water (DI) Required Per Liter}\end{array}$$

c) $$\frac{\text{Total Vol. of MIBAS Sample to be Diluted}}{\text{Proportional Vol. MIBAS Required Per Liter}} = \text{Factor}$$

d) Factor x Proportional Vol. DI Required Per Liter = Total Vol. DI Required to Dilute MIBAS to 26 ^{o}/oo

E.g., dilute MIBAS (1000 ml sample) with initial salinity of 100 ^{o}/oo to 26^{o}/oo.

1. $\frac{26^{o}/oo}{100^{o}/oo} \times 1000 \text{ ml} = 260 \text{ ml}$

2. 1000 ml - 260 ml = 740 ml

3. $\frac{1000 \text{ ml (Vol. MIBAS)}}{260 \text{ ml} = 3.85} = 3.85 \text{ (Factor)}$

4. 3.85 x 740 ml = 2,849 ml DI Required to Dilute a 1000 ml MIBAS Sample to 26 ^{o}/oo.

MIBAS samples generated during the March 1986 land-based tests had an initial salinity ranging from 96^{o}/oo to 120^{o}/oo and an initial pH ranging from 0.0-0.09. Those samples generate required approximately 300 ml of deionized water.

When pH adjustments are made, the salinity will increase to approximately 30 ^{o}/oo.

2. Record the volume of deionized water added (Water Quality Adjustment Form, Section 2). Experience has shown that emission samples generated by the same sampling procedure will require similar adjustment volumes.

3.2.2 Samples of Salinity Less Than 30 ^{o}/oo

1. Add an appropriate volume of concentrated brine to adjust the salinity to 30 ^{o}/oo.

2. Record the volume of concentrated brine added on the Water Quality Adjustment Data Form.

3.3 pH Adjustment

1. Use a series of decreasing NaOH concentrations to adjust pH to 8.0 (± 0.1). The goal is to use the highest concentration possible, to minimize the volume of NaOH solution used, without overshooting the desired pH. Stir the sample and monitor the pH continuously during the pH adjustment process.

2. Add an appropriate volume of 10N NaOH to raise the pH to approximately 7.0.

   MIBAS samples generated during the March 1986 land-based tests required approximately 150-222 ml of 10N NaOH per liter of MIBAS.

3. Add an appropriate volume of 1.0 N NaOH by Eppendorf Pipet (50, 100, or 250 µl) slowly until the pH reaches approximately 7.5.

4. Allow stirring to continue 1-2 minutes and observe stability of the pH. Make minor adjustments with 1.0 or 0.1 N NaOH (µl amounts).

5. If the pH overshoots 8.0, use a minimal amount of 10N HCl, or 1N HCl to readjust back to 8.0.

6. Record the volumes of pH adjusting solutions used (Water Quality Adjustment Data Form, Section 2).

3.4 Final Water Quality Adjustment

1. Add seawater (30 ^{o}/oo) to bring the volume of the sample to within 10 percent of the final volume. (e.g., if diluting to a final volume, add seawater to bring the volume to approximately 9-L).

2. Check salinity and pH. Make appropriate minor adjustments of salinity and pH using minimal amounts of adjusting media. Do not exceed the final adjustment volume (e.g., for a 1000-ml subsample of unadjusted MIBAS, do not exceed 10 liters).

3. Bring the volume of the sample the final volume by addition of seawater (30 ^{o}/oo). E.g., for a 1000-ml subsample, bring to volume of 10 liters.

4. Check salinity and pH. Salinity should be 30 ^{o}/oo (± 1 ^{o}/oo), and pH should be 8.0 (± 0.1). If pH is slightly outside this range, it is possible to readjust with minimal amounts of pH adjusting medium, without significantly changing the volume. Observe the pH after 1-2 minutes to be sure it has stabilized.

5. Record the final salinity and pH of the sample on the label and on the Water Quality Adjustment Data Form (Attachment 1, Section 3).

3.5 Sample Distribution

The final water quality-adjusted sample will be at a 10-percent concentration of the unadjusted emissions sample.

1. While continually mixing, transfer approximately 250-ml of the 10-percent sample to a sample bottle and distribute for *Dinophilus* and/or sea urchin sperm cell toxicity tests.
2. While continually mixing, decant appropriate volumes of the 10-percent sample for the algal (*Champia*), mysid (*Mysidopsis bahia*), and larval fish (*Menidia beryllina*) toxicity tests.

3.6 Glassware Cleaning

Any containers which have come into contact with the sample and are going to be reused during a subsequent sampling period should be cleaned by rinsing three times with deionized water and stored. Just prior to use, rinse the labware with seawater (30^{o}/oo).

4.0 CALCULATIONS

Not Applicable.

5.0 QUALITY CONTROL

A data form (Attachment 2) will be used to document all sample handling procedures for each emissions sample. Data forms will be stored in a three-ring project notebook in the possession of the individual responsible for water quality adjustments during trial incineration burns or at-sea research burns.

6.0 TRAINING

Personnel who will perform this task should first read this SOP and then operate under the supervision of an experienced individual for at least two sampling periods.

7.0 SAFETY

Concentrated NaOH and HCl solutions are used in this procedure and must be handled with extreme care. Also, the emission samples may be very acidic and may contain hazardous components. Safety glasses, gloves, and lab coats must be worn when handling pH adjusting media or emission samples.

8.0 ATTACHMENTS

Attachment 1. Equipment List for Water Quality Adjustments of Aqueous Samples.

Attachment 2. Example of an Emissions Sample Water Quality Adjustment Data Form.

ATTACHMENT 1

EQUIPMENT LIST FOR WATER QUALITY ADJUSTMENTS OF AQUEOUS SAMPLES

Equipment	No. or Amt.	Check
Stir Plate	1	
Stir Bars, 1/2"	4	
Stir Bars, 1"	2	
Stir Bars, 1 1/2"	2	
Stir Bar Retriever	1	
pH Meter (Orion Model 210)	1	
pH Probe (Orion Combination)	2	
pH Meter AC Adapter	1	
pH 4 Buffer Solution	200 ml	
pH 7 Buffer Solution	200 ml	
pH 10 Buffer Solution	200 ml	
ATAGO Salinity Refractometer	1	
Kimwipes	4 Boxes	
Supply of Deionized Water		
Supply of Sitewater		
Supply of Concentrated Brine Solution		
Pipettors, Eppendorf, 50 µl	1	
Pipettors, Eppendorf, 100 µl	1	
Pipettors, Eppendorf, 250 µl	1	
Pipettors, Oxford Macroset (1-5 ml)	1	
Pipette Rack	1	
Pasteur Pipettes, Disposable 5 3/4" Glass	250 (1 Box)	
Pasteur Pipette Bulbs	10	
Pipette Tips for Eppendorfs and Oxford	50 Each	
Volumetric Flask, 2,000 ml	2 (More If Glass)	
Jugs, 1-Gallon	1 Per Sample	
Jars, Wide-Mouth, 2-Liter, Plus Caps	1 Per Sample	
Jars, Wide-Mouth, 250-ml, Plus Caps	1 Per Sample	
Jars. Wide-Mouth, 100 ml, With Caps	4	
Carboy with Spout	2	
Graduated Cylinders, 4,000 ml	2	
Graduated Cylinders, 1,000 ml	2	
Graduated Cylinders, 100 ml	2	
Graduated Cylinders, 50 ml	2	
Graduated Cylinders, 25 ml	2	
Funnels, Large with External Ribs	4	
Squirt Bottles, 500 ml	4	
Flasks, Erlenmeyer, 2,000 ml	4	
Flasks, Erlenmeyer, 1,000 ml	2	
Flasks, Erlenmeyer, 500 ml	2	
Beakers, 1000 ml	2	
Beakers, 250 ml	4	

ATTACHMENT 1 (Continued)

EQUIPMENT LIST FOR WATER QUALITY ADJUSTMENTS OF AQUEOUS SAMPLES

(Continued)

Equipment	No. or Amt.	Check
Sample Labels, Label 1	1 Per Sample	____
Sample Labels, Label 2	1 Per Sample	____
Colored Tape (Red, Pink, White, Plus 1 Other Color)	2 Rolls Each	____
Lab Coat	1	____
Disposable Gloves (Surgical)	1 Box	____
Parafilm	1 Box	____
Sharpies, Pens, Pencils	Several	____
Eyewash Station	1	____
Protective Glasses	1	____
pH Adjusting Solution, 10N NaOH, 500 ml	6	____
pH Adjusting Solution, 1.0N NaOH, 100 ml	1	____
pH Adjusting Solution, 0.1N NaOH, 100 ml	1	____
pH Adjusting Solution, 10N HCl, 500 ml	1	____
NaOH Pellets	2 Kg	____
Paper Towels	Several Rolls	____
Box to Retain pH Adjusting Solutions	1	____

Additional Equipment (At Sea)

"Spill-Proof" Platform
Appropriate Wall Mounts.

Note: The above list is comprehensive, but "extras" of some critical equipment components (i.e. spare pH meter and probe, spare volumetric flask, etc.) should be incorporated into the equipment inventory.

Also, consideration should be given to the prevention of spillage of samples or pH-adjusting media during the at-sea research burns. It may be important to design a "spill-proof" water quality adjustment platform during the next land-based trial burn.

ATTACHMENT 2

Page_________
Date_____________________

EXAMPLE OF AN EMISSIONS SAMPLE WATER QUALITY ADJUSTMENT DATA FORM

Project No.__________________Project Title_______________________________

Refer to SOP No.___________________for Method__________________________

Sample Designation____

1. Preparation

Sample Containers Rinsed (/)	Deionized Water(3x)	Seawater(1x)
1-Gal Jug (for Impinger Sample and Rinses)	__________	________
Container (for Probe Rinses)	__________	________
Container (for Water Quality Adjusted Sample	__________	________
Container (for Water Quality Adjusted Sample)	__________	________

Time Sampling Train Delivered: ______________________________

Impinger Sample, Rinses, and Probe Rinse Combined (/)_____Vol_________

Time Unadjusted Sample Diluted to 4-Liters:__________________________

Salinity of Unadjusted Sample (100-percent MIBAS):________________0/oo

pH of Unadjusted Sample (100 percent of MIBAS):_______________________

Color/Character of Unadjusted Sample:_____________________________

Volume of Aliquot Removed for Water Quality Adjustment:______________

Performed by_________________________Date____________________

---

2. Water Quality Adjustment Data

Time __________ Water Quality Adjustment Began of 100-Percent MIBAS Aliquot.

Salinity Adjustment

Volume Deionized Water Added ______________
Volume Concentrated Brine Added ______________
Volume 30 $^{o}/oo$ Seawater Added ______________

pH Adjustment

Volume of pH Adjusting Medium Added

	NaOH	HCl
10N	______________	______________
1.0N	______________	______________
0.1N	______________	______________

Sample Diluted to Final Volume of ______________ (10% MIBAS)

Comments ____

3. Sample Distribution Data (10% MIBAS Sample)

Final Salinity __________________ Final pH __________________

Sample Properly Labeled (/)

Volume ______ Sample Distributed to

____	sea urchin for sperm test	Time	_____
____	*Dinophilus* Bioassay	Time	_____
____	*Champia* Bioassay	Time	_____
____	Mysid Bioassay	Time	_____
____	*Menidia* Bioassay	Time	_____

Comments ____

Entered by ______________ Date __________ Approved by ______________ Date __________

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF MARINE AND ESTUARINE PROTECTION
INCINERATION-AT-SEA RESEARCH PROGRAM

STANDARD OPERATING PROCEDURES
FOR
CONDUCTING SEA URCHIN SPERM CELL TOXICITY TEST

1.0 OBJECTIVE

The purpose of the sperm cell toxicity test with the sea urchin, Arbacia punctulata, is to determine the concentration of a test substance which reduces fertilization of exposed gametes relative to that of control gametes. Test results are reported as concentration of test substance which reduces fertilization by 50 percent (EC_{50}) or as treatment (or concentration) which produces statistically significantly reduced fertilization. This procedure follows "Methodology and validation of a sperm cell toxicity test for testing toxic substances in marine waters", by P.A. Dinnel, Q.J. Stober, J.M. Link, M.W. Letourneau, W.E. Roberts, S.P. Felton and R.E. Nakatani (Final Report Grant R/TOX-1 for University of Washington, Sea Grant Program in cooperation with the U.S. EPA, 1983) with modifications as developed for conducting sperm cell toxicity tests with Arbacia punctulata at EPA ERL-Narragansett, Rhode Island. The test can be completed within a few hours after sample collection using small volumes (10-100 ml) of seawater and can be performed year round with little advanced preparation in a limited work area.

2.0 TEST PREPARATION

2.1 Test Animals

Gametes from the sea urchin, Arbacia punctulata are used in the Sperm Cell Toxicity Test. Animals can be purchased through the Marine Biological Laboratory, Woods Hole, MA 02543. Arbacia live well in aquaria with running seawater (13-18°C) supplied with Laminaria as a food source. To ensure year-round spawning, animals should be kept at $15 \pm 3^\circ C$. Water quality parameters should be monitored weekly and maintained at $15 \pm 3^\circ C$ and 30 ± 3o/oo salinity. Males and females may be kept in separate compartments for convenience.

2.2 Dilution Water

Seawater (approximately 35 ^{o}/oo) collected from the North Atlantic Incineration Site is diluted with deionized (DI) water to 30^{o}/oo salinity and is referred to here as "site water". It is used in all washing and dilution steps and as control water in the test. Concentrated seawater brine (90-110 ^{o}/oo) is diluted with DI water to 30 ^{o}/oo and is referred to here as "diluted brine." It is used as an additional control treatment in the test. Brine stock is made in large batches by heating to 40°C or less and aerating seawater in large tanks for 3-4 weeks. The advantage of using brine stock to prepare the dilution water is that the quality of this water remains constant from test to test as long as the high quality of DI water remains constant.

2.3 Test System: Equipment

All seawater dilutions, controls, and equipment should be set up prior to sperm collection. Sperm lose their viability rapidly, so it is necessary to work efficiently when making sperm dilutions and to keep the vials of sperm on ice from the time of collection until they are used.

Test concentrations are set up in quadruplicate, using 5-ml volumes in disposable glass scintillation vials. Generally, 50-percent concentration intervals are set up by serially diluting 5-ml volumes of test concentrations with 5-ml volumes of seawater. (See Attachment 2).

2.3.1 Equipment

A list of equipment necessary for conducting this test is given in Attachment 1 (Equipment List for Sperm Cell Toxicity Test).

2.3.2 Solutions

Stock Seawater Solutions

1) Make up 1-2 liters using brine stock with deionized water to 30^{o}/oo. Fill an Accupet dispensor.

2) To calculate the volume of brine which is diluted with DI water to produce 30^{o}/oo seawater, use the formula: $V_B C_B = 30 V_W$ where V_B = volume of brine needed in liters, C_B = concentration of brine in ^{o}/oo, and V_W = final volume of 30^{o}/oo S seawater required.

3) Make up 1-2 liters of site water (30^{o}/oo). Fill a dispensor.

10-Percent Buffered Formalin:

1,620 ml	deionized water
620 ml	formaldehyde (reagent grade)
6.48 g	NaH_2PO_4 or KH_2PO_4 (mono)
10.5 g	Na_2HPO_4 or K_2HPO_4 (dibasic)

Approximately 50 ml needed for each test . Fill the dispensor.

10-Percent Acetic Acid in Seawater

Reagent grade acetic acid with sea water stock. Approximately 50 ml needed. Fill the dispensor.

Test Solution

Adjust salinity of test sample using brine/deionized water to 30^{o}/oo. Record the concentration of test sample in salinity adjusted sample. Record pH.

2.3.3 Seawater Set-Ups

Test Solution

1) Make 50-percent serial dilutions of test solution in quadruplicate with seawater using 5-ml volumes.

See Attachment 2 for diagram of the recommended set up for conducting a serial dilution test.

Sperm Solution

2) Set up sperm dilution series, dispensing 10 ml seawater into one row of vials and pipetting 5 ml seawater into all remaining vials.

See Attachment 2.

2.3.4 Obtain Eggs and Prepare Appropriate Concentration for Test:

1. Select one female and place in shallow bowl, barely covering test with seawater.
2. Stimulate release of eggs from gonopores by touching test with electrodes from 12V transformer (about 30 seconds each time).
3. Collect eggs from between spines using a 10 ml disposable syringe with a large gauge blunt-tipped needle attached. Remove needle before dispensing eggs into 15 ml conical centrifuge tubes.
4. Pool eggs and add seawater to fill tubes. Invert gently several times and gently centrifuge for 1 minute (<1000rpm).
5. Repeat washing procedure.
6. Dilute egg stock using seawater to about 2000 eggs/ml. (See Estimating Egg Concentration, Attachment 3).

2.3.5 Obtain Sperm and Prepare Appropriate Dilution for Test:

1. Select four males and place in shallow bowl, barely covering animals with seawater.
2. Stimulate release of sperm from gonopores by touching test with electrodes from 12V transformer (about 30 seconds each time).
3. Collect 0.5 ml sperm from between spines of each animal using a 1-3 ml disposable syringe with a large gauge blunt-tipped needle attached.

4. Remove needle and place sperm from each animal into four separate glass santillation vials. Keep on ice until used.

5. Dilute sperm from each animal to a concentration of about 8×10^6 sperm/ml or a concentration which will yield fertilization in the range of 60-90 percent. Sperm concentrations are determined with a spectrophotometer which has been zeroed with seawater. (See Estimating Sperm Concentration, Attachment 4).

3.0 TEST PROCEDURES

1. Add 50 µl appropriately diluted sperm from animal No. 1 to one vial of each test concentration (usually set up as one test row). Record time of sperm addition. Sperm should be used within 30 min. of collection.
2. Repeat for sperm samples from each animal (total 4 animals).
3. Incubate all test vials at 20° C for 1 hr.
4. Using a wide mouth pipet tip (one that has had the tip removed with a razor blade), add 1 ml diluted egg suspension to each vial.
5. Incubate 20 minutes at 20° C.
6. Using a dispensor, add 2 ml 10-percent buffered formalin to each sample.
7. Vials may be capped and stored overnight or for several days until evaluated.

4.0 DATA COLLECTION AND TABULATION

1. Transfer 1 ml eggs from bottom of test vials to Sedgwick-Rafter counting chamber. Observe eggs using compound microscope using 10X objective.
2. Count between 100-200 eggs/sample using dual counter.
3. Record total number counted and number unfertilized. Fertilization is defined by the presence of fertilization membrane surrounding egg.

4. Calculate Percent Control Fertilization for each replicate test:

$$\frac{\text{Total No. Eggs} - \text{No. Eggs Unfertilized}}{\text{Total No. Eggs}} \times 100 = \text{Percent Eggs Fertilized}$$

5. Calculate the mean and standard deviation of percent fertilization for those replicate tests considered acceptable (See 5.1 Test Validity) for a particular sample.

6. Tabulate data for EC_{50} calculations:

   a. For acceptable replicate tests, pool (total) the number of eggs counted for each exposure concentration. These data will be entered as the total number of eggs in the EC_{50} program.

   b. For acceptable replicate tests, pool (total) the number of unfertilized eggs counted for each exposure concentration. These data will be entered as the total number of eggs unfertilized in the EC_{50} program.

5.0 DATA ANALYSIS

Data are recorded on standardized data sheets (See Attachment 6). Data are analyzed with the aid of an IBM PC computer using a program written by Dr. Charles E. Stephan, EPA/Duluth in order to generate EC_{50} values and associated confidence intervals. The procedures for data entry and method selection (e.g., bionomial, moving average, or probit) must follow Standard Operating Procedures.

5.1 Test Validity

It is desirable for sitewater control fertilization to be within the range of 60-90 percent and generally this is the primary criterion for acceptability. However, during land-based trial burns, it has been observed that control fertilization improved daily (probably due to recovery from "transport stress" and aquarium acclimation for the sea urchins and resultant improved sperm and egg viability) and the sperm density had to be reduced when it was observed that fertilization exceeded 90 percent in some replicate tests.

It may be necessary during the research burns to conduct initial calibration tests to determine absorbance unit values for a sperm density which will yield 60-90 percent fertilization. It may also be necessary to recalibrate if it is observed that control fertilization exceeds 90 percent. Site water control fertilization outside of the 60-90 percent range may not automatically disqualify a particular replicate test if valuable dose-response data are generated.

6.0 QUALITY CONTROL

Quality control tests can be run using sperm from only one individual and multiple replicates (as many as desired). This procedure allows comparison of controls to be made as well and the diluted brine control fertilization provides a standard control.

7.0 TRAINING

A trainee will conduct the test with a Training Supervisor initially. Determining sperm and egg concentrations and fertilization counts are test specific activities. These functions can be performed independently only after a trainee has demonstrated he or she can accurately reproduce the test to the satisfaction of the Training Supervisor.

8.0 SAFETY

The Sperm Cell Toxicity Test poses little risk to those performing it. Care should be taken when making and dispensing the 10-percent buffered formalin solution; use a hood if available, but make sure the test area is well ventilated. Protective gloves can be worn when pipetting 1 ml of fertilized eggs into the counting cell and rinsing the cell between each sample.

9.0 ATTACHMENTS

Attachment 1. Equipment List for Sperm Cell Toxicity Test

Attachment 2. Diagram of Recommended Setup for Conducting Serial Dilution Test

Attachment 3. Estimating Egg Concentration and Diluting to Appropriate Concentration

Attachment 4. Calibration Curve Correlating Sperm Concentration and Optical Density of Sperm Solutions

Attachment 5. Estimating Sperm Concentration and Diluting to Appropriate Concentration

Attachment 6. Sperm Cell Test Data Sheets

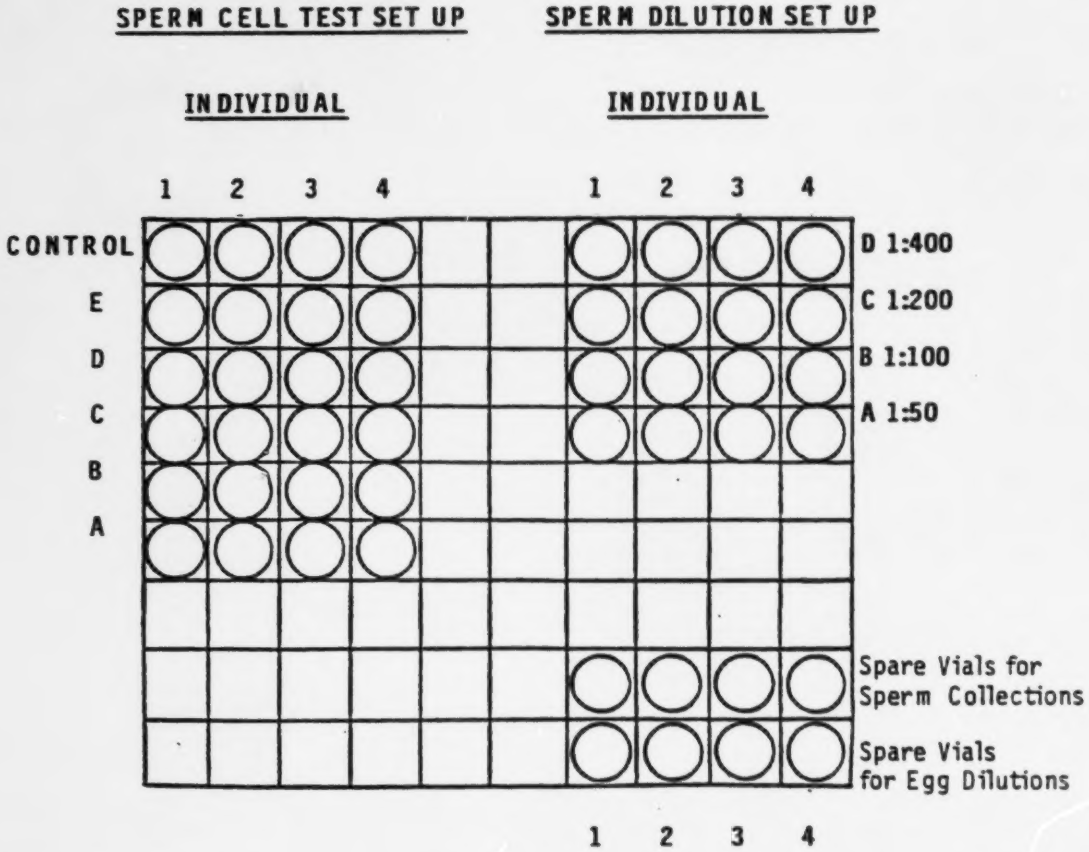
ATTACHMENT 1. EQUIPMENT LIST FOR SPERM CELL TOXICITY TEST

scintillation vials
250 µl, 50 µl, 1000 µl Eppendorf pipettors with tips (1 each)
1 5-ml Macropipettor with tips
3 10 ml Dispensors (for seawater, acetic acid, formalin)
1 5-10cc. Disposable syringe with blunted, large gauge needle
1 1-3cc. Disposable syringe with large gauge needle
Pasteur pipettes with bulbs
1 large Carolina dish
1 Illuminator (desk type)
1 rack to hold syringes, tubes
1 ice bucket w/ice
1 transformer (12V) with electrode
4 male, 1 female Arbacia punctulata
1 centrifuge w/tubes (<1000 rpm)
1 spectrophotometer w/disposable cuvettes
1 compound microscope
1 dissecting microscope
2 glass counting cells (1 ml Sedgwick-Rafter type) and cover slide
transparent counting grid
Aspirator/aeration systems
Several beakers (150-200ml size)
Several graduated cylinders (50-250 ml size)
Manual dual counter, razor blade, paper towels, kimwipes
Labeling tape
Disposable latex gloves
thermometer
parafilm
aluminum foil
fume gard + hood
sandpaper
egg jar + holder

Solutions

Deionized water (> 10 megOhm resistivity)
Brine solution (approximately 120 $^{0}/oo$)
10-percent acetic acid solution (in seawater)
10-percent buffered formalin solution
Filtered seawater at 30 $^{0}/oo$ salinity

ATTACHMENT 2. DIAGRAM OF RECOMMENDED SETUP FOR CONDUCTING SERIAL DILUTION TEST


SPERM CELL TEST SET UP
SPERM DILUTION SET UP
INDIVIDUAL
INDIVIDUAL
1 2 3 4
1 2 3 4
CONTROL
E
D
C
B
A
D 1:400
C 1:200
B 1:100
A 1:50
Spare Vials for Sperm Collections
Spare Vials for Egg Dilutions
1 2 3 4

SPERM CELL TEST 50-PERCENT SERIAL DILUTIONS FOR EACH INDIVIDUAL

ROW A - 5 ml TEST SOLUTION ONLY
ROW B - 5 ml TEST SOLUTION + 5 ml SEAWATER
ROW C - 5 ml OF ROW B SOLUTION + 5 ml SEAWATER
ROW D - 5 ml OF ROW C SOLUTION + 5 ml SEAWATER
ROW E - 5 ml OF ROW D SOLUTION + 5 ml SEAWATER - DISCARD 5 ml
CONTROL ROW - 5 ml SEAWATER

SPERM DILUTION SET UP FOR EACH INDIVIDUAL:

ROW A - 10 ml SEAWATER + .20 ml SPERM (1:50 CONCENTRATION)
ROW B - 5 ml SEAWATER + 5 ml OF ROW A SPERM SOLUTION (1:100 CONCENTRATION)
ROW C - 5 ml SEAWATER + 5 ml OF ROW B SPERM SOLUTION (1:200 CONCENTRATION)
ROW D - 5 ml SEAWATER + 5 ml OF ROW C SPERM SOLUTION (1:400 CONCENTRATION) - DISCARD 5 ml

ATTACHMENT 3

ESTIMATING EGG CONCENTRATION AND DILUTING TO APPROPRIATE CONCENTRATION

1. Add seawater to washed eggs to volume of 100 ml ("egg stock").

2. Mix egg stock using a 3-pronged glass tube (1mm ID) air jetting device. Using wide mouth pipet tip, transfer 1 ml eggs from egg stock to vial containing 9 ml SW. (This vial contains an egg suspension diluted 1:10 from egg stock).

3. Mix vial using gentle pipetting. Using wide mouth pipet tip, transfer 1 ml eggs from vial to Sedgwick-Rafter counting chamber. Count all eggs in chamber using dissecting scope with objective at 12X ("egg count").

4. Calculate concentration of egg stock (no. eggs/ml) = 10 times "egg count". Dilute egg stock to 2000 eggs/ml by formula below.

   a. If eggs counts >=200:
      "egg count" / 200 = Y
      (Y x 200) - 200 = Volume seawater to add to egg stock in ml

   b. If egg count <200, obtain more eggs, wash as previously described. It is helpful to save a reserve stock of washed eggs during the collection procedures. Add to egg stock and begin counting and diluting procedure again.

5. Transfer 1 ml from diluted egg stock to vial containing 9 ml SW. Mix, then transfer 1 ml from vial to Sedgwick-Rafter counter. Count all eggs using dissecting microscope. Confirm final egg count = 200/ml.

ATTACHMENT 4

CALIBRATION CURVE CORRELATING SPERM CONCENTRATION AND OPTICAL DENSITY OF SPERM SOLUTIONS

1. Make sperm dilutions of 1:100, 1:200 and 1:400 using glacial acetic acid in seawater. Read absorbance of sperm solution spectrophotometrically (540nm):

   Add 200 ul sperm to 19.8 ml, 10-percent glacial acetic acid in seawater (solution a). Mix by inversion. Subsample 5 ml from solution a, add 5 ml seawater (solution b). Mix by inversion. Subsample 5 ml from solution b, add 5 ml seawater (solution c). Mix by inversion. Read absorbance of solutions a, b and c using spectrophotometer and cuvettes intended for routine testing.

2. Dilute sperm solutions as necessary to obtain accurate sperm cell counts using hemacytometer. Calculate sperm concentrations for all solutions:

   a. Add 1 drop sperm solution c to Neubauer hemacytometer chamber. Let settle 15 minutes then count sperm in central 400 squres, using a compound microscope (400 x). Calculate dilution factor for solution c that would give a hemacytometer count of about 50. Dilute subsample of solution c accordingly (solution c') and take duplicate sperm cell counts of this solution. Calculate sperm concentration in solution c' by the following equation:

      sperm/ml = average hemacytometer count x 10(4)

   b. Repeat counting process for subsamples of solutions a and b, diluted by dilution factor calculated above (solutions a' and b'). Calculate sperm concentrations for solutions a, b, c, a', b', c' using equation and dilution factors.

3. Repeat process for ten different sperm samples.

4. Correlate sperm concentrations and absorbance readings to determine absorbance reading which represents desired sperm concentration for toxicity testing:

   Generate table of values for sperm concentrations (calculated from hemacytometer counts) and corresponding absorbance readings. Plot sperm concentration (x) vs. absorbance units (y). Use linear regression analysis to generate best-fit line. Solve for absorbance reading corresponding to 8x10(6) sperm/ml.

ATTACHMENT 4 (Continued)

EXAMPLE:

1.

Absorbance Readings, AU	Solution	Dilution Factor from original sample = d
0.144	a	100
0.072	b	200
0.036	c	400

2.a. Preliminary hemacytometer count of solution c: 363
b. Dilution factor necessary to make hemacytometer count of solution c' = 50: 363/50 = d'

HEMACYTOMETER SPECTROPHOTOMETER

Solution Average	Hema. Ct.	Calc. Sperm Conc.	xd'	=	Sperm Conc.	Solution
a'	207.0	2.07x10(6)	x7	=	1.45x10(7)	a
b'	101.0	1.01x10(6)	x7	=	7.10x10(7)	b
c'	51.4	5.14x10(5)	x7	=	3.60x10(6)	c

3.a. Table values:

Sperm Sample	Solution	Sperm Conc.	SPM(*)	AU540
1	a	1.45x10(7)	1.45	0.144
1	b	0.71x10(7)	0.072	
1	c	0.36x10(7)	0.36	0.036

* SPM = sperm/mlx10(-7)

b. Best line: SPM = (9.61) * AU540 + (0.05)
c. Desired sperm concentration = 8x10(6) sperm/ml = 0.80 SPM
(0.80 - 0.05)/ 9.61 = 0.079
desired absorbance reading = 0.079

ATTACHMENT 5
ESTIMATING SPERM CONCENTRATION AND DILUTING TO APPROPRIATE CONCENTRATION

FOR EACH SPERM SAMPLE:

1. Label vials and add seawater as specified:

   A. 10 ml
   B. 5 ml
   C. 5 ml
   D. 5 ml

2. Add 200 µl sperm to Vial "A" using wide pipet tip. ("A" contains 1:50 dilution original sperm concentration).

3. Mix "A" by gentle pipetting using 5 ml pipetter. Transfer 5 ml from "A" to Vial B. Discard 2.5 ml from Vial A. ("B" contains 1:100 dilution of original sperm concentration).

4. Mix "B" by gentle pipetting using 5 ml pipetter. Transfer 5 ml from "B" to Vial C. ("C" contains 1:200 dilution of original sperm concentration).

5. Mix "C" by gentle pipetting using 5 ml pipetter. Transfer 5 ml from "C" to Vial D. ("D" contains 1:400 dilution of original sperm concentration).

6. Mix "D" by gentle pipetting using 5 ml pipetter. Discard 5 ml from "D". (Note: Volume in all vials is now 5 ml).

7. Add 5 ml (10 percent) Glacial acetic acid seawater to Vial C. ("C" contains 1:400 dilution of original sperm concentration--as does Vial D. Vial C contains killed sperm). Cap Vial C and mix by inversion. Transfer about 2 ml from "C" to spectrophotometer cuvette.

8. Read cuvette at 540nm ("AU") using seawater as blank. Calculate predicted AU for Vials A, B, and D (containing live sperm):

VIAL	PREDICTED AU
D	= C
B	= 4 x C
A	= B x C

9. Select Vial (A, B, or D) with predicted AU >= value determined by calibration curve (see Attachment 4). Dilute selected vial to final predicted AU by adding seawater accordingly:

   Predicted AU for selected vial /Target AU = Y
   (Y x 5) - 5 = Volume of seawater to add to selected vial in ml.

EXAMPLE: AU for Vial C = 0.030

VIAL	PREDICTED AU	
D	= C	= 0.030
B	= 4 x C	= 0.120
A	= B x C	= 0.240

Select Vial B, dilute as below:

ATTACHMENT 6

SPERM CELL TEST DATA SHEETS

SPERM CELL TEST (Arbacia punctulata)

GAMETES

TEST ID____________________ INITIALS__________

SPERM

COLLECTION TIME:________ TARGET SPERM DENSITY______ AU DATE__________

	AU540/DILUTION	DILUTION FOR TEST	CALCULATED SPERM DENSITY	TIME IN
1.				
2.				
3.				
4.				

EGGS

COLLECTION TIME:__________

INITIAL COUNT/VOLUME:__________

FINAL COUNT:__________

TEST TIMES

	SPERM IN	EGGS IN	FORMALIN IN
1.			
2.			
3.			
4.			

ATTACHMENT 6 (Continued)

SPERM CELL TEST DATA SHEETS

SPERM CELL TEST (Arbacia punctulata)

SAMPLE

TEST ID____________________ INITIALS__________

COLLECTION DATE:____________________

SALINITY/ADJUSTMENT:____________________

pH/ADJUSTMENT:____________________

PHYSICAL CHARACTERISTICS:____________________

COMMENTS:____________________

TEST CONCENTRATIONS:____________________

CONTROL WATER:____________________

TEST TEMPERATURE:____________________

ATTACHMENT 6 (Continued)

SPERM CELL TEST DATA SHEETS

SPERM CELL TEST (*Arbacia punctulata*)

DATA

TEST ID INITIALS

SAMPLE DATE

STATISTICAL ANALYSIS

EC_{50} (95-Percent CI):

METHOD CALCULATED:

COMMENTS:

ANIMAL NUMBER	1 Total/Unfert	% F	2 Total/Unfert	% F	3 Total/Unfert	% F	4 Total/Unfert	% F
CONCENTRATION								
ACCEPT/REJECT								

% F = Percent Fertilized.

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Page 1 of 10

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF MARINE AND ESTUARINE PROTECTION
INCINERATION-AT-SEA RESEARCH PROGRAM

STANDARD OPERATING PROCEDURE FOR CONDUCTING STATIC-RENEWAL RAPID CHRONIC TOXICITY TESTS WITH Mysidopsis bahia

1.0 OBJECTIVE

The purpose of the 7-day static-renewal bioassay with Mysidopsis bahia is to determine the best estimate of the concentration of a test material which causes adverse effects to mysids during the seven-day test period. Test results are reported as the concentrations at which a statistically significant difference (P=0.05) from the control treatment occurred for survival and specific chronic effects.

2.0 PREPARATION

2.1 Test Animals

2.1.1 Culturing of Mysidopsis bahia for Toxicity Testing

All mysids used in a test must be from the same brood stock, which must have been cultured for at least one generation using the procedures outlined in the 1985 ASTM Draft No. 8 of "Proposed New Standard Practice for Conducting Life-Cycle Toxicity Tests with Saltwater Mysids." Brood stock can be obtained from another laboratory, a commercial source, or a wild population from an unpolluted source.

2.1.2 Feeding and Handling

Mysid culture stocks and test animals are fed ad libitum, at least once daily, Artemia sp. nauplii no more than 24 hours post-hatch, at a ration of 150 Artemia nauplii per mysid per day. New batches (lots) of Artemia are analyzed according to the 1985 ASTM Draft No. 2 of "Proposed New Standard Practice for the Use of Brine Shrimp

Nauplii as Food for Toxicity Test Organisms" and are not fed to mysids in a culture or test until it has been shown that the batch will support survival, growth, and reproduction of the test species.

2.1.3 Diseased Animals

Mysids that exhibit abnormal behavior or appear sluggish or diseased must not be used in testing or used as parental stock to obtain juvenile mysids.

2.1.4 Acquisition of Juvenile Mysids

Only 7-day-old mysids must be used for rapid-chronic testing. Brood stock should be obtained from cultures of similar conditions (i.e., 30 ± 2°/oo and 26 ± 1°C). Eight days before the proposed test start date, sufficient females with eyed eggs in the brood sacs are stocked in brood chambers to be assured of a sufficient yeild. Approximately the same number of gravid females should be harvested as juveniles required for the test.

The temperature in the brood chambers can be increased to the upper acceptable culture limit (27°C). Within 24 hours, sufficient juveniles should be available for the test. The juveniles are removed from the brood chambers (day 0) and placed in a separate tank for holding. Test animals are held for six days, during which time they are acclimated to the test conditions, if necessary. The holding temperature is critical to development during this period, and should be maintained at 26 ± 1°C. On the sixth day, they are transported to the test site.

2.2 Dilution Water

Tests are conducted using natural clean seawater or a dilution of brine and deionized water. Salinity should be adjusted using brine or deionized water. There should be controls using natural seawater with adjusted salinity, and brine and deionized water, if that is used to prepare test solutions, or water from the test site, if that is used in the treatment concentrations.

2.3 Description of Test System

2.3.1 Test Chambers and Aquaria

Plastic (polystyrene) disposable 200-ml test cups are used for the static-renewal tests. Cups are pre-soaked in clean flowing seawater at least overnight before testing. Test cups are held in plastic trays, with pre-drilled holes for stability, in a constant temperature water batch during the test. Generally, five concentrations plus one or two controls are tested with 5 animals/cup and at least 8 cups/concentration.

2.3.1.1 Acceptability

Holding, acclimation, and test chambers which will support healthy growth of mysids are considered acceptable for use in testing.

2.3.2 Environmental Conditions

Water quality for mysids during rapid-chronic testing will be maintained within the following ranges:

Temperature	26 ± 2°C
Salinity	30 ± 2°/oo
Dissolved Oxygen	>4.2 mg/l
Light	16L:8D (With 30 Min. Phase-In/Out Period)

3.0 TEST PROCEDURES

3.1 Test Initiation

The rapid-chronic test is started by placing forty, 7-day-old juvenile mysids in each test concentration and the control(s). Animals for each concentration are randomly assigned to eight replicate test cups, five per cup. The number of mysids present is checked immediately after dispensing and recounted within one hour. Test solutions (150-ml volume) are renewed daily by decanting off 90 percent of the old solution and gently adding the fresh solution.

4.0 DATA COLLECTION

4.1 Routine Biological Monitoring

Mysids in each replicate cup are monitored daily. Dead mysids are counted, removed, and recorded at this time. Excess food is removed at the time of test solution renewal. Each replicate is fed 24-hr post-hatch Artemia nauplii daily, after test solutions are renewed.

Biological observations should include: number dead, number immobilized, number missing, and erratic, sluggish, or other abnormal behavior. Missing mysids should be recorded separately, but considered "dead" in data analysis. In addition, the presence of turbidity, precipitates, or any other unusual condition (smell, fumes) should be noted. All observations should be recorded on the data sheet (Attachment 2).

On the last day of the test, each cup is observed for the number of survivors, their sex, the number of immature animals, and the presence or absence of eggs in the oviducts or brood sacs of the females. These data are recorded on the data sheet.

After determining the number of gravid females, all survivors are placed by replicate in 4-percent buffered formalin or another non-destructive preservative to determine mean weights. All mysids from each group are rinsed with deionized water, then placed (using forceps) on a tared weigh pan (one pan per replicate). They are then dried for at least 24 h at 60°C to attain a constant weight. The weigh boats are transferred immediately from the oven to a desiccator, then weighed to the nearest microgram. These data are recorded on the data sheet (Attachment 1) and the mean dry weights per individual are calculated.

4.2 Routine Test Monitoring

4.2.1 Water Quality

The monitoring schedule for water quality parameters is detailed below: The measurements will be made according to written SOPs and the data recorded on standardized data sheets (Attachment 2).

Water Quality Parameter (Units)	**Monitoring Frequency**	**Units Measured**	**Treatment Monitored**
Dissolved Oxygen (ppm)	Daily	Nearest 0.1	All Treatments (At Least 2 Replicates)
Salinity (^{0}/oo)	Daily	Nearest 0.5	All Treatments (At Least 2 Replicate)
Temperature (^{0}C)	Daily Hourly	Nearest 0.5 Nearest 1.0	All Treatments (At Least 2 Replicates

5.0 DATA REDUCTION AND REPORTING

At the end of the mysid chronic bioassay, the concentrations producing significant mortality should be reported. The toxic endpoint is expressed as an effect concentration if adverse effects (such as loss of equilibrium or immobilization) were observed in addition to mortality. References for the methods used to analyze the data should be reported.

The number of immature animals and the number of females with eggs in the oviducts or brood sac should be reported for each concentration by replicate and as the mean and standard deviation of all replicates. The number of immature animals should also be reported for each concentration as the percentage of the total number of animals surviving to the end of the test. The number of females with eggs in the oviducts or brood sac should also be expressed for each concentration, as the percentage of the total number of females surviving to the end of the test. The presence or absence of egg development in females should be statistically analyzed on arcsine root-transformed data by analysis of variance and Duncan's multiple range test using SAS computer programs.

The mean, standard deviation, and ranges are reported for: temperature, salinity, and dissolved oxygen. These calculations are based on the routine samples scheduled for measurement during the test (Section 4.2.1).

Temperature	Rounded to the nearest 0.5°C
Salinity	Rounded to the nearest 0.5°/oo
Dissolved Oxygen	Rounded to the nearest 0.1 ppm

6.0 QUALITY CONTROL

In order for the test to be acceptable, survival must be at least 80 percent in the control treatment at the termination of the test. Dissolved oxygen should not fall below 4.2 ppm anytime during the test. Salinity should not exceed 34°/oo, and temperature should be maintained at 26 ± 2°C throughout the test. Data input for computer analysis will be double-checked for accuracy with the original data set.

7.0 TRAINING

A trainee must be thoroughly familiar with these Standard Operating Procedures and will be monitored in the conduct of this test by a Training Supervisor, initially. The test can be run independently only after the trainee has demonstrated that he or she can accurately perform all aspects of the test in the judgement of the Training Supervisor.

8.0 SAFETY

There are no special safety precautions associated with the performance of this test.

9.0 ATTACHMENTS

Attachment 1.	7-Day Rapid Chronic Mysid Test Biological Data Sheet
Attachment 2.	7-Day Rapid-Chronic Mysid Test Water Quality Data Sheet
Attachment 3.	7-Day Rapid Chronic Mysid Test Summary Data Sheet

ATTACHMENT 1. 7-DAY RAPID-CHRONIC MYSID TEST BIOLOGICAL DATA SHEET

SITE: ______________________

START DATE: ______________________

SALINITY: ______________________

TEMPERATURE ______________________

Treatment/ Replicate	Pan No.	Tare Wt.	Total Wt.	Animal Wt.	No. of Mysids	X Wt./ Individuals

SITE: ______________________

START DATE: ______________________

SALINITY: ______________________

TEMPERATURE ______________________

EFFLUENT CONC./REP.	DAY 1 NO. ALIVE	DAY 2 NO. ALIVE	DAY 3 NO. ALIVE	DAY 4 NO. ALIVE	DAY 5 NO. ALIVE	DAY 6 NO. ALIVE	DAY 7 NO. ALIVE	NO. MATURE FEMALES	NO. IMMATURE ANIMALS	INITIALS
CONC.										
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
D.O.										
SALINITY										
TEMP.										
pH										
CONC.										
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
D.O.										
SALINITY										
TEMP.										
pH										
CONC.										
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
D.O.										
SALINITY										
TEMP.										
pH										
CONC.										
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										

ATTACHMENT 3. 7-DAY RAPID-CHRONIC MYSID TEST SUMMARY DATA SHEET

SITE: ______________________________
START DATE: ________________________
SALINITY: __________________________
TEMPERATURE: _______________________

Treatment	
No. Live Larvae	
Survival (percent)	
Females with Eggs (percent)	
Mean dry Wt./ Larvae (mg) ± S.D.	
Mean Temperature (°C) ± S.D.	
Mean Salinity (°/oo) ± S.D.	
Av. Dissolved Oxygen (mg/L) ± S.D.	

Comments:

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Page 1 of 17

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF MARINE AND ESTUARINE PROTECTION
INCINERATION-AT-SEA RESEARCH PROGRAM

STANDARD OPERATING PROCEDURES

FOR

CONDUCTING SEVEN-DAY GROWTH/SURVIVAL (STATIC RENEWAL) STUDIES WITH THE INLAND SILVERSIDE, *Menidia beryllina*

1.0 OBJECTIVE

The purpose of the *Menidia beryllina* seven-day growth/survival study is to measure the effects of toxicants using growth and survival as endpoints. Larval *Menidia beryllina* are exposed to several concentrations of a toxicant in a static toxicity test, with daily renewals for seven days. The results of this test are reported as those concentrations which cause growth or survival to be statistically significantly ($p=0.05$) different from the control and concentrations where differences could not be detected.

2.0 PREPARATION

2.1 TEST ANIMALS

2.1.1 Source and Handling

Menidia beryllina may be cultured in the laboratory or obtained from Gulf or Atlantic coast estuaries throughout the year. Gravid individuals can be collected from low salinity waters during April to July depending on latitude along the Atlantic coast. *Menidia beryllina* are very sensitive to handling and should never be removed from the water by net, but should be transferred only by beaker or other container. Feral brood stock are preferred to minimize inbreeding although fish have been reared to maturity in the laboratory within 6 to 8 months after hatching if they are held in flowing seawater at 25-30°C and salinity 20-35 o/oo, with a photoperiod of 12 to 14 h. Adults should be fed flake food or frozen brine shrimp twice a day and live *Artemia* nauplii once per day. Tanks should be cleaned weekly.

2.1.2 Obtaining Embryos and Maintaining Embryos and Larvae

Embryos can be obtained by natural spawning on aquarium floss that is placed in the tanks for 24 hours. Floss should be removed from tanks and placed in aquaria for hatching. Embryos incubated at 25°C and 30 o/oo salinity hatch in 6 to 7 days. Newly hatched larvae are fed rotifers for approximately five days post-hatch, at which time the diet is changed to newly hatched *Artemia* nauplii (about 24 hours post-hydration). The one-week post-hatch larvae are ready for use in conducting a Fish Growth/Survival Study.

2.1.3 Feeding

The five day old *Menidia* larvae can be fed *Artemia* nauplii (about 24 hours post-hydration). Larvae must receive nutritious portions of food daily throughout the seven day exposure period because one of the endpoints of the test is growth.

The procedure for hatching *Artemia* is as follows:

1. Fill a 2- to 4-liter separatory funnel (or other appropriate container) with 20-25°C seawater insure adequate hatching. Add 10 cc/liter brine shrimp cysts, and aerate for at least 24 h at 25°C. (Two separatory funnels are recommended, each started on alternate days, since it may require more than 24 h to hatch certain strains of brine shrimp.).

2. Nauplii will hatch from brine shrimp cysts within 24 to 48 h in aerated, 25°C seawater, but before nauplii are fed to fish or mysids,

they should be separated from the cysts using one or more of the following procedures: 1) the phototactic response of the nauplii, 2) sieves, 3) density differences between cysts and nauplii.

3. After removing the source of air, the nauplii's phototactic response is utilized by covering the funnel with a dark cloth or paper towel for 5 min, allowing nauplii to concentrate at the bottom. Leaving nauplii much longer than 5 min without aeration may cause mortality.

4. To segregate nauplii from the cysts: Rinse the nauplii into a beaker (500 ml) or a black separator box (15 x 8 x 8 inches high), place a light source at one end, and leave for no more than 10-15 min. After live nauplii and unhatched cysts have separated, pipette or siphon off the live nauplii.

5. Pour the nauplii onto a Nitex screen (mesh <150 μm), rinse with filtered 25 to 30 ^{o}/oo seawater, and drain off most of the water.

6. Resuspend nauplii in seawater to a constant volume (250 to 500 ml), enumerate the nauplii or otherwise estimate the concentration per volume seawater, and adjust appropriately (refer to mysid or fish guidance manuals). The nauplii may now be fed to mysids or fish larvae in the appropriate amounts.

2.1.4 *Artemia* Quality Control

Before use, individual lot numbers of cysts should be fed to the test organisms in 7-day studies to confirm diet is adequate for the purpose of the test. The shelf-life of an opened container of cysts may be affected by humidity and temperature. However, as long as >90 percent of the cysts hatch in 24-48 hours and the control responses are acceptable, the cysts may be used.

2.2 DILUTION WATER

Tests are conducted using natural clean seawater or a dilution of brine and deionized water. Salinity should be adjusted using brine or deionized water. There should be controls using natural seawater with adjusted salinity, and brine and deionized water, if that is used to prepare test solutions, or water from the test site, if that is used in the treatment concentrations.

2.3 DESCRIPTION OF TEST SYSTEM

2.3.1 Test Chambers

Test chambers for the Menidia beryllina larvae growth/survival test should be adequate to hold at least 50 ml of test solution/larvae; 20 larvae/replicate. The fathead minnow chamber has been used successfully and extensively for this study. The design was developed by Norberg and Mount (in press) and is constructed with glass, mesh and silicone. Alternatives to several features were introduced: 1) 200 μm nylon mesh was used instead of stainless steel mesh; and 2) thin pieces of glass have been affixed with silicon to the nylon mesh as reinforcement across the bottom and at the sides and middle.

Use of the fathead minnow chamber is recommended as it is easily constructed, holds an adequate volume and incorporates a sump area which leaves the larvae relatively undistrubed as the test solution is siphoned off or renewed. However, each chamber must be used as a replicate. One to two liters of test solution may be added per chamber. A total of 2 liters of test solution in each chamber will usually insure that evaporation will not cause salinity to rise more than 2 ^{o}/oo between solution renewals.

Use of other glass chambers that hold an adequate volume (e.g., crystallization dishes) and incorporate a sump area is also feasible. The depth of the test solutions in the testing chambers should be at least 5 cm.

2.3.2 Experimental Design

Each test should consist of five concentrations and a control, with three replicates per concentration, 15 larvae/replicate.

Selection of larvae for each treatment and positioning of treatments and replicates should be conducted randomly.

2.3.3 Test Parameters

1. Test type: static, with 24-h renewal
2. Salinity: 5 ^{o}/oo-30 ^{o}/oo ± 2 ^{o}/oo
3. Temperature: 25 ± 2°C
4. Dissolved Oxygen: > 60 percent saturation
5. Photoperiod: 14 h light, 10 h dark
6. Light intensity: minimum of 100 ft candles
7. Light quality: Covered soft-white light
8. Test chamber size: Glass dish at least 8.0 cm high, adequate to contain test solution volume to a height of ≥5.0 cm, and having a sump area.
9. Test solution volume: 50 ml/larva (approximately 1L/replicate)
10. Renewal of test solutions: Daily
11. Age of test organisms: 5-7 days post-hatch
12. Number of treatments per study: 5 treatments and a control
13. Number of test organisms per test chamber: 15 larvae/replicate
14. Number of replicate chambers per treatment: 3
15. Source of food: ≤24-28 hour old *Artemia* nauplii

16. Feeding regime: Feed 0.10 g *Artemia* nauplii per replicate on days 0-2. Feed 0.16 g *Artemia* nauplii per replicate on days 3-6

17. Cleaning: Siphon daily, before renewal and feeding

18. Aeration: None, unless DO concentration falls below 3.5 mg/l, then aerate *all* test chambers

19. Dilution Water: Uncontaminated source of seawater, deionized water mixed with hypersaline brine or artificial sea salts.

20. Test duration: 7 days

21. Effects measured: Survival and growth

2.3.4 Test Equipment

The equipment necessary to perform this test is listed in Attachment 1.

3.0 TEST PROCEDURES

3.1 Test Initiation

Using a wide bore pipette with an opening ≥3 mm, deliver larvae randomly to each replicate of each concentration, two or three at a time, until there are 15 larvae/replicate and 45 larvae/concentration. *Menidia beryllina* are active and practically swim out of the eyedropper when it is immersed in the test solution. By allowing the larvae to swim out of the pipette into the test solution or gently dispensing the larvae with only a small amount of the seawater contained in the eyedropper, the dilution of the test solutions is minimized. If the chambers are compartmentalized, the larvae should be apportioned equally between the compartments.

Test solutions are renewed daily (see Section 4.1).

3.2 Chamber Randomization

Each replicate chamber should be randomly distributed in the temperature-controlled water bath. Treatments should be chosen blindly to determine the order of the first replicate. Place the treatments in a row as determined by this selection process and repeat until all chambers are in place. Maintain this configuration throughout the test. A location chart may be helpful.

3.3 Environmental Controls

Place chambers in a stable environment where the temperature can be maintained at 25 ± 2°C. A temperature controlled water bath, room or incubator may be used.

a. If a water bath is used, the depth of the water surrounding the chambers should be a minimum of 2.5 cm.

b. Rooms or incubators with high volume ventilation should be used with caution as volitization of the test material and/or wide fluctuations in salinity may occur.

The light intensity at the surface of the test solutions should be bright enough to promote adequate larval growth. A minimum of 10 ft candles should be measured at the surface of the test solutions. 100-125 ft candles has been employed effectively. Covered white light is recommended for safety reasons and for its diffusive properties.

Measure and record temperature, salinity and dissolved oxygen concentration of each replicate (pages 1 and 2 of data sheets, Attachment 2).

a. The temperature should be maintained at 25 ± 2°C and should not vary more than 1°C between chambers on a given day.

b. The salinity should be maintained at (30 ^{0}/oo) and should not vary more than 2 ^{0}/oo between chambers on a given day.

c. The oxygen meter used during the test should be calibrated against a standard Winkler or mini-Winkler titration, prior to taking dissolved oxygen levels in the test solutions. This procedure should be repeated before each study. The measurements should be taken after the test solutions are poured into the chambers.

4.0 DATA COLLECTION AND TEST MAINTENANCE

4.1 BIOLOGICAL AND WATER QUALITY MONITORING

Days 1-6

1. Measure temperature, salinity, and dissolved oxygen concentrations once per day and record (pages 1 and 2 of data sheets, Attachment 2). Measure dissolved oxygen at least twice during days 1-6. On these days dissolved oxygen should be measured before the solutions are renewed, i.e. after 24 hours.

2. Make up test solutions.

3. Put chamber on light table and count live larvae.

4. Remove dead larvae and record the number of live larvae on data sheets (pages 1 and 2, Attachment 2).

5. Siphon off 90 percent of test solution from each chamber into a large beaker.

   a. Attempt to siphon off all *Artemia* from previous day from each chamber. (If chambers are placed on light table for 5-10 minutes prior to siphoning, the *Artemia* will concentrate and be easier to siphon).

   b. Siphon remainder of test solution from sump area.

c. Check for larvae in large beaker containing solution just siphoned. Gently return any larvae by eyedropper to appropriate relicate and record the number and location of siphoned larvae in case handling mortality results.

6. Slowly refill each chamber with appropriate amount of seawater or toxicant solution, without disturbing the larvae. Renewals should be poured into the sump area using a narrow bore (approximately 9 mm ID) funnel.

7. Feed new Artemia to larvae in each replicate (as outlined above). Return the chamber to the water bath.

On day seven (test termination) do the following:

1. Measure temperature, salinity and dissolved oxygen concentrations and record (pages 1 and 2 of data sheets. Attachment 2).

2. Count and record live larvae on data sheets (pages 1 and 2, Attachment 2).

3. Remove dead larvae, if necessary.

4. Determine the number of surviving larvae/treatment and conduct an ANOVA (as described under Data Reduction and Analysis) to determine which treatments are significantly different in survival from the control(s). Surviving larvae in treatments that are significantly different from the control(s) need not be weighed.

5. Siphon live larvae from individual replicates, through a 505-μm mesh screen to remove fecal material and Artemia. Rinse larvae with deionized water to remove salts.

6. At this point larvae can be preserved in premarked vials containing a 4 percent formalin solution for future dry weight analysis. When ready to perform the dry weight measurements, remove the larvae from the formalin, place on a screen and rinse with deionized water.

7. Pieces of aluminum foil or small aluminum weigh boats can be used for dry weight analysis. Foils or pans should not exceed 10 mg in weight. Aluminum foil, approximately 1.5 cm x 1.0 cm and 5.0 - 7.0 mg in weight, has been used without difficulty.

8. Weigh pieces of aluminum foil or small weigh boats (1/replicate), record weight, and place in larger aluminum weigh boats marked for identification (page 3 of data sheets, Attachment 2).

9. After completing either Step 5 or 6 (above), use forceps to place the larvae from one replicate briefly on a paper towel to dry.

10. Using forceps, place total number of larvae from one replicate on a preweighed foil or weigh boat

    a. Record the number of larvae for each dry weight on the data sheets (pages 1 and 3, Attachment 2).

    b. Return foil or small weigh boats to the larger marked weigh boats.

    c. Repeat Steps 7 and 8 until all replicates are completed.

11. Place weight boats in drying oven for a minimum of 2 hours at 60°C.

12. Transfer immediately from the oven to a dessicator.

13. Reweigh all foils or weigh boats and determine final dry weight of the replicate to 0.01 mg. Record on page 3 of data sheets (Attachment 2).

14. Divide the final dry weight per replicate by the number of larvae on the foil or weigh boat to determine the mean individual dry weight per replicate. Record on pages 1 and 3 of the data sheets (Attachment 2).

15. Complete the summary data sheet (page 4 of Attachment 2) after calculating the average measurements and statistically analyzing the dry weights and percent survival.

5.0 DATA REDUCTION AND ANALYSIS

A One-Way Analysis of Variance (ANOVA) is conducted on survival data to determine significant (alpha = 0.05) differences from the control. Convert the number surviving/replicate for each treatment to a proportion of the total exposed/replicate. Use the arcsine root transformation on the proportions, then perform a One-Way Analysis of Variance and a Duncan's multiple range test on these data. Determine if survival in any treatment is significantly less than control survival. Perform a One-Way Analysis of Variance and Duncan's multiple range test on the mean individual dry weights and determine which are significantly different (alpha = 0.05) from the control mean individual dry weight. The estimated No Observed Effect Concentration (NOEC) can then be determined, if desired, from the more sensitive endpoint(s).

6.0 QUALITY CONTROL

A test should not be considered definitive if the average survival of control fish is less than 85 percent and the mean 7-day control dry weight is less than 0.43 to 0.5 mg/fish if the fish are preserved or not preserved, respectively. Dissolved oxygen should not be permitted to fall below 3.5 mg/l during the test. Temperature during the test should be maintained at 25°C ± 2°C. At any time during the test, temperature between treatment chambers should not vary by more than 1°C and salinity should not vary more than 2°/oo.

7.0 TRAINING

The trainee must first be thoroughly familiar with this SOP. The trainee will be monitored by a Training Supervisor through all aspects of the test. The test can be run independently only after he or she has demonstrated to the satisfaction of the Training Supervisor that they are capable of meeting the quality control criteria and accurately reproducting the measurements necessary to perform the test.

8.0 SAFETY

There are no special safety precautions associated with the performance of this test. Care should be taken when handling the 4-percent formalin solution.

9.0 REFERENCES

Ablett, R.F. and R.H. Richards. 1980. Suitability of twenty-four-hour and forty-eight-hour unfed Artemia as an early foodstuff for "0" group Dover sole (Solea solea L.) production. Aquaculture, 19:371-377.

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10.0 ATTACHMENTS

Attachment 1. Equipment List for Conducting Test

Attachment 2. Data Forms for Fish Growth/Survival Study

ATTACHMENT 1. EQUIPMENT LIST FOR CONDUCTING TEST

6 beakers of 2 liter or more for test solutions
Two - 1 liter graduated cylinders (one clean and one contaminated)
Two - 250 ml graduated cylinder (one clean and one contaminated)
20 aluminum foils/test in marked weigh boats (2 extra)
Forceps
Large bore pipettes
Desiccator
19 - 2 liter chambers/test (includes 1 extra)
Colored tape
Water-proof markers
Minimum of 400 embryos per test
Air lines
Air stones
Silent Giant Pump
Two 2 or 4 liter separatory funnels for *Artemia*
Artemia (brine shrimp) cysts
Mesh seives (150 and 505m)
One - 5 ml pipette with siphon bulb (for feeding)
Squirt bottle for deionized water
Dissecting Microscope
Siphon with bulb and clamp
3 liter beakers for test solution drainage
Light table
Data sheets (one set/test)
Protocol
Dissolved oxygen meter
Refractometer
20 scintillation vials containing 4-percent buffered formalin
Standard or micro-winkler apparatus

ATTACHMENT 2. DATA FORMS FOR FISH GROWTH/SURVIVAL STUDY

Type Effluent ______________________ Field __________ Lab ________ Test __________

Effluent Tested: ____

CONCENTRATION:

	REPLICATE:								REPLICATE:							
DAYS	0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7
# LIVE LARVAE																
TEMP. (°C)																
SALINITY ($^{0}/_{00}$)																
D.O. (mg/l)																
# LARVAE / DRY WEIGHT				MEAN WEIGHT / LARVAE (mg) ± S.D.					# LARVAE / DRY WT.				MEAN WEIGHT / LARVAE (mg) ± S.D.			

CONCENTRATION:

# LIVE LARVAE																
TEMP. (°C)																
SALINITY ($^{0}/_{00}$)																
D.O. (mg/l)																
# LARVAE / DRY WEIGHT				MEAN WEIGHT / LARVAE (mg) ± S.D.					# LARVAE / DRY WT.				MEAN WEIGHT / LARVAE (mg) ± S.D.			

CONCENTRATION:

# LIVE LARVAE																
TEMP. (°C)																
SALINITY ($^{0}/_{00}$)																
D.O. (mg/l)																
# LARVAE / DRY WEIGHT				MEAN WEIGHT / LARVAE (mg) ± S.D.					# LARVAE / DRY WT.				MEAN WEIGHT / LARVAE (mg) ± S.D.			

COMMENTS:

TIME FED							

FISH GROWTH / SURVIVAL STUDY

Test Dates:____________________ Species:____________________

Pan #	Conc. & Rep.	Initial Wt. (mg)	Final Wt. (mg)	Diff. (mg)	# Larvae	Av. Wt./ Larvae (mg)	Pan #	Conc. & Rep.	Initial Wt. (mg)	Final Wt. (mg)	Diff. (mg)	# Larvae	Av. Wt./ Larvae (mg)

FISH GROWTH / SURVIVAL STUDY

Test Dates: ____________________ Species: ____________________

Original Effluent: pH ____________ Salinity ____________ Age of Larvae: ____________

CONCENTRATION:																
REPLICATE:									**REPLICATE:**							
DAYS	0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7
# LIVE LARVAE																
TEMP. (°C)																
SALINITY ($^{0}/_{00}$)																
D.O. (mg/l)																
# LARVAE / DRY WEIGHT				MEAN WEIGHT / LARVAE (mg) ± S.D.					# LARVAE / DRY WT.				MEAN WEIGHT / LARVAE (mg) ± S.D.			
CONCENTRATION:																
# LIVE LARVAE																
TEMP. (°C)																
SALINITY ($^{0}/_{00}$)																
D.O. (mg/l)																
# LARVAE / DRY WEIGHT				MEAN WEIGHT / LARVAE (mg) ± S.D.					# LARVAE / DRY WT.				MEAN WEIGHT / LARVAE (mg) ± S.D.			
CONCENTRATION:																
# LIVE LARVAE																
TEMP. (°C)																
SALINITY ($^{0}/_{00}$)																
D.O. (mg/l)																
# LARVAE / DRY WEIGHT				MEAN WEIGHT / LARVAE (mg) ± S.D.					# LARVAE / DRY WT.				MEAN WEIGHT / LARVAE (mg) ± S.D.			

COMMENTS:

Summary of Test Conditions
FISH GROWTH / SURVIVAL STUDY

Test Dates: ______________________ Species: ______________________

Effluent Tested: ______________________

TREATMENT						
# LIVE LARVAE						
SURVIVAL (%)						
MEAN DRY WT. / LARVAE (mg.) ± S.D.						
SIGNIF. DIFF. FROM CONTROL (*)						
MEAN TEMPERATURE (°C) ± S.D.						
MEAN SALINITY ‰ ± S.D.						
AV. DISSOLVED OXYGEN (mg./L) ± S.D.						

COMMENTS:

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF MARINE AND ESTUARINE PROTECTION
INCINERATION-AT-SEA RESEARCH PROGRAM

STANDARD OPERATING PROCEDURES

FOR

ESTIMATING CHRONIC SEXUAL REPRODUCTION EFFECTS WITH *CHAMPIA PARVULA*

1.0 OBJECTIVE

This standard operating procedure describes a method which uses sexual reproduction, after a short-term exposure to a toxicant, to estimate chronic toxicity. In brief, the method consists of exposing male and female *Champia parvula* to exposure media for two days, followed by a 5- to 7-day recovery period in control medium. The recovery period allows time for any cystocarps to mature. At the end of the recovery period the number of cystocarps per plant are counted. The objective of this test is to use absence of sexual reproduction as the endpoint. Statistical differences, although more difficult to interpret ecologically, are also included to facilitate comparisons with other marine species.

2.0 PREPARATION

2.1 Test Organism

Plants of *Champia parvula* (C. Agardh) Harvey (Rhodophyta) are bushy and 5 to 10 cm tall in the field. The main axis and branches are cylindrical, hollow and septate. *Champia's* life history is an alternation of isomorphic generations (Figure 1). The clone presently used was isolated in Rhode Island in 1979. It is probably not essential to have a standard clone, however, some experience is required to isolate new clones from the field into unialgal culture. Unialgal stock cultures are necessary to maintain healthy, actively growing plants for use in testing. Vegetatively propagated plant material from the 1979 clone is available from the U.S. Environmental Protection Agency, South Ferry Road, Narragansett, RI 02882.

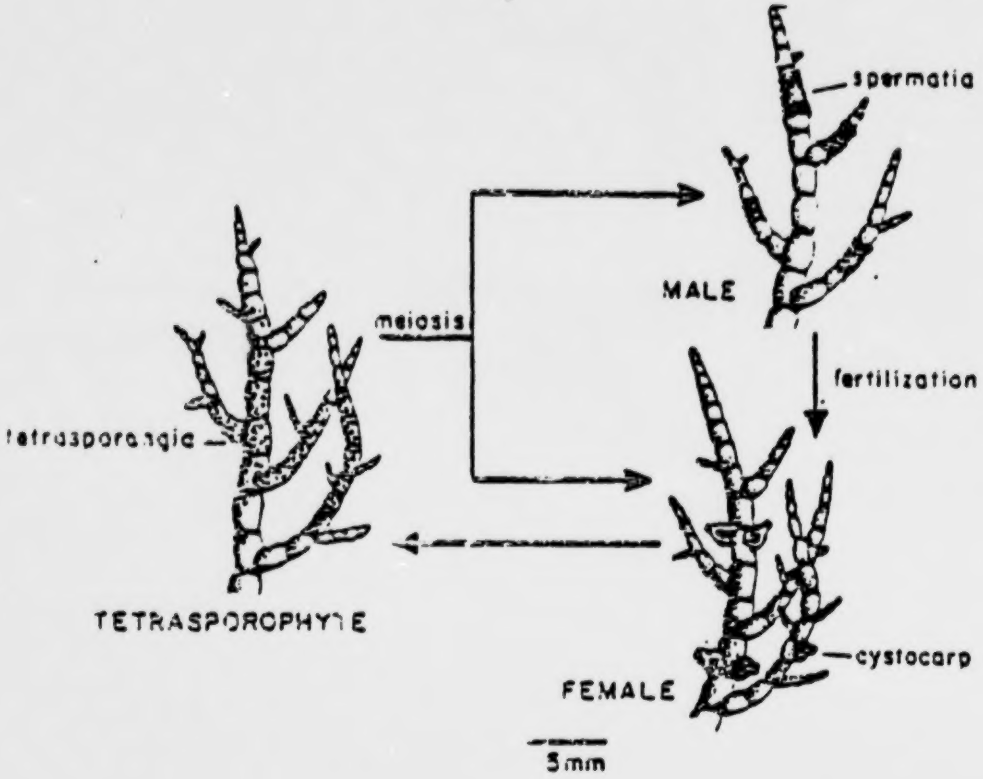

spermatia
MALE
meiosis
fertilization
tetrasporangia
TETRASPOROPHYTE
cystocarp
FEMALE
5mm

Figure 1. Life history of the marine red alga Champia parvula.

2.1.1 Maintenance of Stock Cultures of *Champia parvula*

The equipment necessary to perform the test is listed in Attachment 1. Male and female plants of *Champia* can be maintained easily in unialgal culture in the laboratory. New cultures can be started from excised branches, making it possible to maintain clonal material indefinitely. No special preconditioning is required to induce reproduction. Under the conditions listed below, male gametophytes produce spermatia continuously and females are always receptive. Thus, plant material can be available at any time for testing.

Laboratory cultures of *Champia* provide test plants of similar preconditioning. Unialgal stock cultures of both males and females are maintained in separate, aerated 1000-mL Erlenmeyer flasks containing 800 mL of the culture medium. The culture medium is made from natural seawater to which additional nutrients are added (Table 1). The seawater is autoclaved for 30 min at 15 psi. The culture flasks are capped with aluminum foil and autoclaved dry, for 10 min. Culture medium is made up by dispensing seawater into the sterile flasks and adding the appropriate nutrients from a sterile stock solution. Alternately, liter flasks could be autoclaved with the seawater already in them. Sterilization is used to prevent microalgal contamination, and not to keep cultures bacteria-free.

Several cultures of both males and females should be maintained simultaneously to keep a constant supply of plant material available. Some cultures should be at different stages of development (i.e., with different amounts of tissue per flask). Initial stock cultures should be started weekly with about twenty 0.5 to 1.0 cm branch tips. Cultures are gently aerated through sterile, cotton-plugged, disposable, polystyrene 1 mL pipets. Cultures are capped with foam plugs and aluminum foil and illuminated from the side with 75 $\mu E\ m^{-2}\ s^{-1}$ of cool-white fluorescent light on a 16:8, light:dark cycle. The temperature is 22 to 24^{o}C and the salinity 28 to 30 $^{o}/oo$. Media are changed once each week.

About half of the plants should be discarded (or placed into another culture vessel) with each weekly medium change to reduce the amount of biomass as the plants grow. At the end of three weeks plants will be ready to use for testing. Readiness is

Table 1. Recipe for Additional Nutrients to be Added to Natural Seawater for *Champia parvula* Stock Cultures and Test Medium. Both EDTA and Trace Metals have been Omitted. The Concentrated Stock Solution is Autoclaved at Standard Temperature and Pressure for 15 Minutes.

	Amount/Liter	
COMPOUND	Final Solution	Concentrated Stock[a]
$NaNO_3$	9.35 mg	3.74 grams
$NaH_2PO_4H_2O$	0.62 mg	0.25 grams
Iron[b]	2.6 µg	1.04 mg
Vitamins		
B_{12}	0.06 µg	10 mL[c]
Biotin	0.06 µg	
Thiamine · HCl	12.5 µg	

[a] Use 0.2 mL/80mL (2.5 mL/L) for test medium concentrations and 10 mL/L for stock cultures. For test medium only, add 0.2 mL/80mL of a sodium bicarbonate solution. A stock solution of 60 mg/mL sodium bicarbonate is prepared by autoclaving it as a dry powder and then dissolving it in sterile deionized water.

[b] Iron stock solution prepared by dissolving 1 g iron powder in 10 mL concentrated HCl and diluting to 1 liter with deionized water. Acceptable stock solutions could also be made with ferric or ferrous chloride.

[c] Vitamin stock solution autoclaved separately in 10 mL sub-samples. Each 10 mL contains 24 µg biotin ---, 24 µg B_{12},--- and 5 mg thiamine-HCl.

defined as having enough plant material to perform at least one test. With this procedure, actively growing plants will be continuously available. The total number of cultures maintained will depend on the expected frequency of testing.

2.1.2 Unhealthy Plants

A stock culture should not be used as a source of test material if the plants appear to be stressed or undernourished. Under conditions of stress the tips of the branches will turn "pink" and the older tissue will generally be much paler. Under conditions of nutrient deficiency (resulting usually from too much plant material in the culture flask or too long since the last medium change) the entire plant will turn pale yellow. If the stress is severe enough the older tissues (main axes) or occasionally the branch tips will turn white (evidence of necrotic tissue). If cystocarps are present on females in the stock cultures, the plants are not suitable for testing (this usually happens as a result of contamination by males or water from male cultures).

2.2 Artificial Seawater

Because salinity adjustments are necessary in testing most complex effluents, an artificial seawater recipe that yields good growth of Champia is desirable. An artificial seawater would also make the test method more readily available to laboratories that do not have access to clean natural seawater. Some commercial preparations are toxic to Champia (presumable due to the presence of high concentrations of trace metals that are in the commercial grade of salts used in their preparation). EPA-ERL-Narragansett has had initial success with artificial seawater using Provasoli's (1963) ASP-1, although the morphology of the thallus of Champia was not entirely satisfactory in this medium. Recently, another artificial seawater, GP2 (Spotte, et al., 1984), has shown promise for meeting the test needs (plants grow and reproduce well and have the correct morphology). However, comparisons between the sensitivity to toxicants in this medium with that in natural seawater have not yet been conducted. Work is in progress to minimize the amount of EDTA that is necessary to add to GP2. When this is determined the above comparison can be made. Until then, use of artificial seawater medium for culture or testing is not recommended.

3.0 PROCEDURE

3.1 Readiness of Stock Plants

Stock cultures should be checked for their readiness for use in toxicity tests. Females can be checked by examining a few branch tips under a compound microscope (100 X or greater). Several trichogynes (reproductive hairs to which the spermatia attach) should be easily seen near the apex (Figure 2). Male plants should be visibly producing spermatia. This can be checked by placing some male tissue in a petri dish, holding it against a dark background and looking for the presence of spermatial sori (Figure 3). Another way is to examine the males under a compound microscope, where a mature sorus can be easily identified by looking at the edge of the thallus (Figure 4). A final, quick way to determine the relative "health" of the male stock culture is to place a portion of a female plant into some of the water from the male culture for a few seconds. Under a compound microscope numerous spermatia should be attached to both the sterile hairs and the trichogynes (Figure 5).

3.2 Preparation of Plants for a Test

Once cultures are determined to be usable for toxicity testing, branch tips should be cut into their final size. For females, cut 7 to 10 mm branch tips, enough for 5 per treatment chamber (try to be consistent in the degree of branching; see Figure 6). The cutting can be easily done with fine-point forceps with the plants in a little seawater in a petri dish. Repeat for males, except cut 1.5 to 2 cm branches and only one per treatment chamber. The males should visibly be producing spermatia (i.e. two or more spermatial sori present). Cut the females first to minimize the chances of contaminating them with water from the male stock cultures.

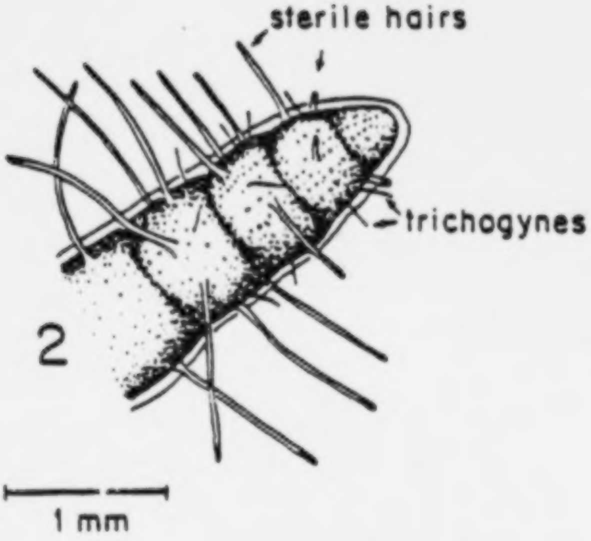


Figure 2. Apical tip of female branch of Champia parvula showing sterile hairs and trichogynes (reproductive hairs). Sterile hairs are wider and generally much longer than trichogynes. They also appear to be hollow except at their apex. Both types of hairs occur around the entire circumference of the thallus but are seen easiest at the "edges". Receptive trichogynes occur only near the branch tips.

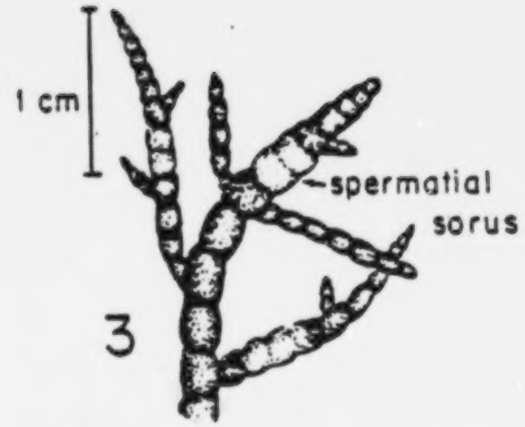


Figure 3. A portion of a male thallus of Champia parvula showing spermatial sori. The sorus areas are generally slightly thicker in diameter and a little lighter in color.

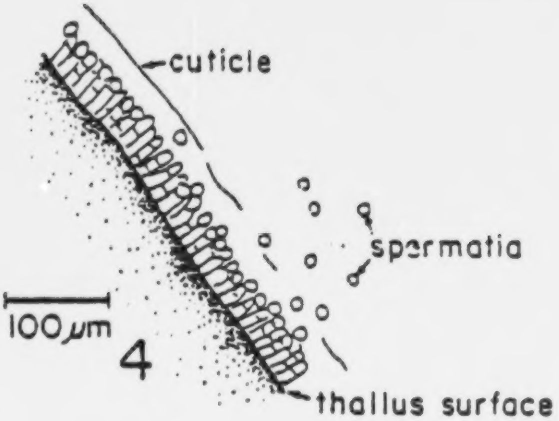
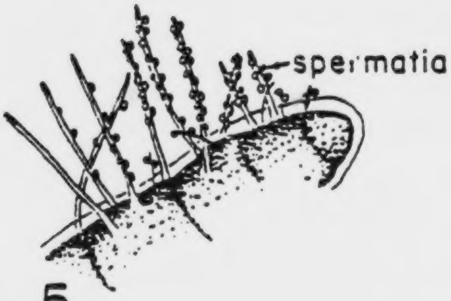

cuticle
spermatia
100 µm
4
thallus surface

Figure 4. A close-up of a portion of a spermatial sorus, note the rows of cells that protrude from the thallus surface.



5

Figure 5. Apical tip of a female of Champia parvula that had been "dipped" in water from a male culture. The sterile and reproductive hairs are covered with spermatia. Note that older hairs (those more than about one mm back from the apex) have few to no spermatia attached to them.

6

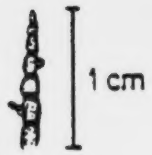


Figure 6. The size and degree of branching that is generally used for the female starting plants. Occasionally the branches will be longer near the tip, try to be consistent in the degree of branching since the receptive trichogynes are at the branch tips. Therefore, the more variation in the degree of branching, the more variation in the potential number of cystocarps per plant.

3.3 Protocol

1. Set up and label control and treatment chambers; three per treatment and controls.

2. Fill chambers with 80 mL of control or treatment water. Alternately, all chambers can be filled with control water and the toxicant added with micropipets.

3. Add 0.2 mL of both the nutrient and bicarbonate stock solutions to each chamber (see Table 1).

4. Add five female branch tips and one male branch to each chamber.

5. Place chambers under cool-white light (ca 75 $\mu E\ m^{-2}\ s^{-1}$) timed fora light:dark cycle of 16:8. Place a thermometer in a flask of water among the chambers.

6. Gently hand-swirl chambers twice a day (spermatia are not motile, therefore water motion is critical). Alternately, shake continuously at 100 rpm on a rotary shaker. Record temperature daily in °C and salinity in $^{o}/oo$.

7. If desired, media can be changed after one day.

8. After 2 days:

   a. Label recovery bottles (these can be almost any type of container or flasks with 200 to 400 mL of natural seawater plus the additional nutrients (see Table 1).

   b. With forceps, gently remove females from test chambers and place into recovery bottles. Add aeration tubes and foam stoppers.

   c. Place bottles under the same cool-white light as the stock cultures and aerate for the 5- to 7-day recovery period.

4.0 DATA COLLECTION

At the end of the recovery period count the number of cystocarps per female and record the data (Attachment 2). Cystocarps are counted by placing females between the inverted halves of a polystyrene petri dish with a small amount of seawater (to hold the entire plant in one focal plane). Using a stero-microscope, the emergent cystocarps can be easily counted. Cystocarps are distinguished from young branches because they possess an apical ostiole (opening for spore release) and darkly pigmented spores (see Figures 7 and 8). One of the advantages to this test procedure is that if there is uncertainty about the identification of an immature cystocarp, then the plants can just be aerated for a little longer in the recovery bottles. No new cystocarps will form since the males have been removed, the plants will only get bigger.

5.0 DATA ANALYSIS

Chronic values are calculated using two criteria for determining significant differences. One criterion for difference from controls is absence of cystocarps (<5 percent of the control). The other criterion is based on statistical differences. Statistically significant decreases in the number of cystocarps are determined by one-way analysis of variance (ANOVA) followed by Dunnett's mean separation test (alpha = 0.05) for comparison of treatments with a control (Steel and Torrey, 1960). The results from each replicate chamber are reported as the mean number of cystocarps per plant (n=3) for each treatment rather than for statistical purposes. If one chamber is dropped or otherwise lost, then there are still observations at that treatment concentration. Chronic values are expressed either as the no-effect range, the lowest concentration that results in a significant difference from the control and the next lowest concentration tested, or as the geometric mean of these two values (Buikema, et al., 1982). If the lowest concentration that results in a significant difference is the lowest concentration tested, then the geometric mean is not calculated. One can only report the lowest concentration that gave the significant difference.

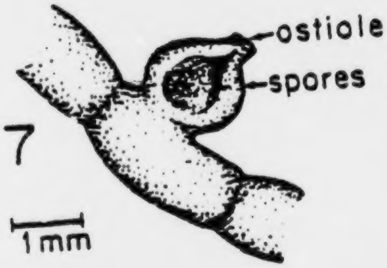


Figure 7. A mature cystocarp of Champia parvula. In the controls and the lower effluent concentrations, cystocarps are often clustered in groups of as many as 10 to 12.

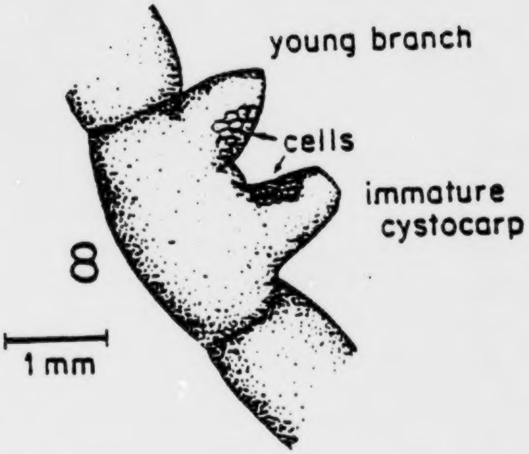


Figure 8. Comparison of a very young branch with an immature cystocarp. Both can have sterile hairs and the young branch may or may not have trichogynes. However, the immature cystocarps will never have trichogynes. Young branches are usually more pointed at the apex and will not form an ostiole. The cell dimensions of young branches are larger than those of the cystocarp.

6.0 QUALITY CONTROL

1. A test is not acceptable if control mortality exceeds 20 percent.
2. If plants fragment in either the controls or the lowest exposure concentration so that individual plants can not be identified, then the test is not acceptable. This is not critical if absence of sex is the only endpoint of interest. However, the fact that the plants fragmented indicates they are not at their best and the data may be biased toward the lower concentrations.
3. A test should not be considered definitive if the controls average fewer than 10 cystocarps per plant. If no sexual reproduction occurs in the controls, then this test can not be considered acceptable.
4. The data from the two replicates at the concentration determined to be the effect concentration should be statistically equal (i.e. there should not be a significant difference ($p < 0.05$) between the two control replicates determined by a t-test).

7.0 TRAINING

A trainee must be thoroughly familiar with this standard operating procedure and will be monitored in the conduct of the test by a Training Supervisor, initially. The test can be run independently only after the trainee has demonstrated that he or she can accurately perform all aspects of the test in the judgement of the Training Supervisor.

8.0 SAFETY

There are no special safety precautions associated with the performance of this test.

9.0 REFERENCES

Buikema A.L., Niederlehner B.R., Cairns J. 1982. Biological monitoring, Part Iv, Toxicity Testing. Water Res. 16: 239-262.

Provasoli L. 1963. Growing marine seaweeds. Proc. Int. Seaweed Symp. 4:9-17.

Spotte S., Adams G., Bubucis P.M. 1984. GP2 medium is an artificial seawater for culture or maintenance of marine organisms. Zoo. Biol. 3:229-240.

Steel R.G., Torrey J.H. 1960. Principles and procedures of statistics. McGraw Hill. 481 pp.

10.0 ATTACHMENTS

Attachment 1.	**List of materials for performing toxicity tests with _Champia parvula_**
Attachment 2.	**Sample data sheet for number of cystocarps per plant of _Champia parvula_**

Author Approval: ________________, ________________

________________, ________________

Technical Director Approval: ________________, ________________

QA Approval: ________________, ________________

Laboratory Director Approval: ________________, ________________

Name **Date**

ATTACHMENT 1

LIST OF MATERIALS FOR PERFORMING TOXICITY TESTS WITH Champia parvula.

1. Fine-point stainless steel forceps.
2. 100-mL polystyrene cups with covers (or 125-mL Erlenmeyer flasks) to be used as exposure chambers.
3. Polystyrene petri dishes to hold plants for counting cystocarps and for cutting branch tips.
4. 100-mL graduated cylinders to measure control and treatment volumes.
5. 1 and 10-mL disposable pipets.
6. Digital micropipets (200 and 1000 µl maxima) if dilutions are made directly in test chambers.
7. Disposable tips for micropipets.
8. Nutrient and sodium bicarbonate stock solutions (see Table 1).
9. Recovery bottles or flasks, need one per treatment or control chamber.
10. Aquarium pump(s) and air tubing.
11. Plastic aeration tubes (1-mL disposable pipets work fine) and foam plugs.
12. Thermometer and flask or bottle to hold it.
13. Marking pens and colored tape.
14. Cool-white fluorescent lighting, sufficient to give 75 $\mu E\ m^{-2}\ s^{-1}$ (ca 500 foot-candles) on a timer with a light:dark cycle of 16:8.
15. Rotary shaker for exposure chambers (hand-swirling twice a day can be substituted).
16. Stereomicroscope for counting cystocarps.
17. Refractometer for salinity measurements.
18. Data sheets (one per test).
19. Protocol.

ATTACHMENT 2

SAMPLE DATA SHEET FOR NUMBER OF CYSTOCARPS PER PLANT OF *Champia parvula*

EFFLUENT____________________ **INITIALS**________________

Exposure began on (date)________________

Recovery began on (date)________________

Counted on (date)________________

REPLICATES	CONCENTRATIONS (%)						
	Control						
A1							
2							
3							
4							
5							
MEAN							
B1							
2							
3							
4							
5							
MEAN							

Temperature: day 0________, day 1________, day 2________ °C

Initial Salinity:________Percent

Source of dilution water:

Light conditions:

Comments:

SOP No. 3-10
Revision No. 01
Date of Revision: 15 May 1986
Page 1 of 10

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF MARINE AND ESTUARINE PROTECTION
INCINERATION-AT-SEA RESEARCH PROGRAM

STANDARD OPERATING PROCEDURES FOR

LIFE-CYCLE TOXICITY TESTS WITH *Dinophilus gyrociliatus*

1.0 OBJECTIVE

The purpose of the 7-day rapid chronic bioassay with *Dinophilus gyrociliatus* is to determine any adverse effect of a test material on any stage of the life-cycle of this archiannelid worm.

Both mortality data and sublethal reproductive effects (e.g., eggs produced/female) can be used as endpoints. This SOP has been adapted from the Final Report submitted to EPA on "Evaluation of the Archiannelid *Dinophilus gyrociliatus* for Use in Short-Term Chronic Bioassays" authored by Carr and Curran, 1985.

2.0 TEST PREPARATION

2.1 Test Animals - Culture Maintenance and Feeding

A subculture of *Dinophilus gyrociliatus* was originally received in May, 1984, from Dr. Bertil Åkesson of the University of Gothenburg, Sweden. Cultures of *Dinophilus gyrociliatus* are maintained in millipore (0.45 μm) filtered, aerated, unsterilized, 25 ⁰/oo salinity seawater at 20 ± 2°C in one-liter glass jars with screw-top lids at Battelle New England Marine Research Laboratory (Duxbury, MA). Filtered compressed air supplied at a "slow bubble" rate through narrow bore tygon tubing with a glass Pasteur pipet at the terminal end provides circulation in the jar. Cultures are fed approximately 5.0 ml of a 0.5 percent suspension of frozen fragmented spinach every 2-3 weeks. Cultures should be reestablished every 2-3 months by placing 50-100 individuals in fresh cultures as described above. A new culture should be sufficiently well-established to supply enough animals for a complete test from a single jar after 3 weeks from the time of reestablishment.

The salinity of cultures should be checked twice weekly and adjusted to 25 ± 1^{0}/oo if necessary. Adjustments and comments are recorded on standardized forms in the Dinophilus culture maintenance notebook which is kept in the culture room.

2.2 Animal Handling and Collection

When a culture is ready for harvesting either for testing purposes or for culture reestablishment, the same procedure is used. Approximately 800 ml is slowly siphoned off into a clean container from an undisturbed culture. Care should be taken to avoid siphoning off any particulate matter from the bottom or sides of the jar. The remaining contents of the jar are then gently swirled in order to suspend the animals in the water column and the contents are decanted into a glass finger bowl for inspection. The animals are removed from this bowl with a Pasteur pipet with the aid of a dissecting microscope and transferred to a smaller dish containing fresh filtered seawater. If animals are being collected for a test, then 1-2 day-old animals are placed in a separate container and the older animals are generally used to reestablish another culture.

2.3 Glassware Cleaning

All glassware is washed according to a specified procedure initially and after contact with any contaminated solutions. Glassware which only comes in contact with seawater or uncontaminted solutions is recleaned by three rinses with deionized water and then air-dried.

2.4 Food Preparation

Frozen spinach is initially shaved from a frozen block with a glass microscope slide. The spinach is dried at 70°C, ground with a mortar and pestle and then passed through a 105-micron sieve. This powdered spinach is stored in a cool dry location in a screw-capped jar. For a test or culture feeding, a 0.5-percent solution of this powdered spinach is prepared in seawater and stirred on a magnetic stirrer to ensure homogeneity among sample aliquots.

2.5 Test Equipment

The equipment list necessary to perform this test is given in Attachment 1.

3.0 TEST PROCEDURES

3.1 Test Setup

The tests are conducted in 20-ml stender dishes with ground glass lids with 10 ml of solution per dish. Four animals are placed into each dish with 5 dishes/concentration. If brine/deionized water seawater is used as the diluent, than both brine and natural seawater controls (25 ^{0}/oo S) are run in conjuction with toxicant concentrations which are usually made up as 50 percent serial dilutions with 3-5 concentrations tested.

3.2 Test Initiation

The test is started with one to two-day-old animals. An experienced investigator can easily differentiate between newly released juveniles and more mature animals because they grow so rapidly. The test solutions are first dispensed to the exposure chambers. The animals are collected as described in Section 2.2 and placed individually into the chambers using a Pasteur pipet with a latex bulb. All observations and manipulations are performed using a dissecting microscope with fiber optic illumination. After the animals have been added, each chamber is reexamined to verify that there are at least 4 animals/replicate at the start of the test. After the chambers have been reexamined, 50 µl of a 0.5 percent spinach solution is dispensed to each dish. Observation for survival, egg production, and juvenile release are made daily. Eighty percent of the solution in each chamber is renewed daily by withdrawing 8 ml of solution with an Oxford-type pipetor while examining the dish under a dissecting scope, to ensure that no eggs or animals are inadvertently removed, and then adding 8 ml of a freshly prepared solution.

4.0 DATA COLLECTION

4.1 Water Quality Data

On day 1 and 6 of the test, temperature, dissolved oxygen, salinity, and pH measurements will be made on the renewal solutions and pooled replicate solutions collected from each dish during the renewal procedure for each treatment (i.e., a 24-hour old sample). These measurements will be made according to Standard Operating Procedures and the data will be recorded on standardized data sheets (see Attachment 2).

4.2 Biological Data

Each chamber is examined every 24 hours before the solutions are renewed. Survival and reproductive data for each chamber are recorded on standardized data sheets (see Attachments 3 and 4, respectively). The eggs of Dinophilus gyrociliatus are sexually dimorphic with the female eggs much larger than the males. There are generally 2-5 eggs/egg case with the majority of the eggs being female. Because the males die shortly after copulation, which occurs in the egg case, only female eggs are used in the egg production counts. The first eggs are usually laid on day 3 or 4 with the present protocol. New juveniles may begin to emerge by day 5 or 6. The daily reproductive data recorded for each chamber are the total number of female eggs, the number of egg cases, and the number of newly emerged juveniles. At the termination of the test, the number of eggs visible in the coelom of each animal will also be recorded.

5.0 DATA ANALYSIS

LC_{50} estimates will be generated using a statistical program written by Charles E. Stephan, EPA/Duluth, Minnesota. Reproductive data will be analyzed by a multiple range test (e.g., Duncan's, Dunnett's, or Student-Newman-Keuls) with the aid of SAS to determine significant differences between the treatments and the controls.

6.0 QUALITY CONTROL

All raw data will be printed out with the computer programs. The hard copy printout will be rechecked with the raw data in order to insure accurate data transcription.

7.0 TRAINING

A trainee will set up test chambers and practice daily monitoring under a experienced Training Supervisor initially. The test can be run independently only after the trainee has demonstrated he or she can accurately reproduce counts at the judgement of the Training Supervisor. Any questions concerning the test should be directed to Dr. R. Scott Carr, of Battelle New England Marine Research Laboratory (Duxbury, MA).

8.0 SAFETY

There are no special safety precautions associated with the performance of this test.

9.0 ATTACHMENTS

Attachment 1. Equipment List for *Dinophilus* Life-Cycle Test

Attachment 2. Example of *Dinophilus* Water Quality Monitoring

Attachment 3. Example of *Dinophilus gyrociliatus* Toxicity Monitoring Sheet

Attachment 4. Example of *Dinophilus gyrociliatus* Reproduction Data Sheet

ATTACHMENT 1

EQUIPMENT LIST FOR Dinophilus LIFE-CYCLE TEST

Glass Stender dishes with ground glass lids (approximately 20-ml size)
Dissecting microscope with illuminator (fiber optics is suggested)
Plastic or wooden tray
Pasteur pipets (with latex bulbs)
5-ml Oxford-type macropipettor (with tips)
25 or 50-microliter pipettor (with tips)
2-3 small Carolina dishes
Millipore filtering apparatus (with 0.45-μm filters)
Vacuum pump
Rubber tubing
2 150-ml beakers
1,000-ml filtering flask
1,000-ml Erlenmeyer flask
Colored labeling tape
Sharpies and pens
Data sheets
Manual counter
Kimwipes
Parafilm

For Food Preparation:

Frozen spinach
glass slide
Watch Glass
Oven
Teflon tissue grinder (with glass tube)
150-μm sieve
Mortor and pestle
Turkey baster (optional)

For Culture of Animals:

1-liter glass jar (with lid)
Tygon tubing to fit Pasteur pipet
Clean Air supply

ATTACHMENT 2

Dinophilus gryociliatus WATER QUALITY MONITORING

Test Sample________________________________Project No.________________

Burn No.____________________Date Start____________Finish________________

Concentration of Adjusted Sample________________________Initials________

Comments____

____

		Day						
Conc.	Rep.	1	2	3	4	5	6	7
CONTROL	A							
	B							
	C							
	D							
	E							
	A							
	B							
	C							
	D							
	E							
	A							
	B							
	C							
	D							
	E							
	A							
	B							
	C							
	D							
	E							
	A							
	B							
	C							
	D							
	E							

ATTACHMENT 3

Dinophilus gryociliatus TOXICITY MONITORING SHEET

Test Sample______________________________ Project No.____________________

Burn No.__________________________________ Date________________________

Concentration of Adjusted Sample____________________ Initials______________

Comments____

____

TEST CHAMBER

Temperature (°C)								
Salinity (°/oo)								
pH								
Dissolved Oxygen (mg/l)								
Saturated DO (ppm)								
% Saturation DO								
*Test Medium Character								
Comments								

TEST CHAMBER

Temperature (°C)								
Salinity (°/oo)								
pH								
Dissolved Oxygen (mg/l)								
Saturated DO (ppm)								
% Saturation DO								
*Test Medium Character								
Comments								

*CLDY	Cloudy
PRE	Precipitate
FUS	Film on Surface
UN	Undissolved Chemical
PM	Particulate Matter

Temperature Ranges	Low________	High________
Salinity Ranges	Low________	High________
pH Ranges	Low________	High________
Dissolved Oxygen Ranges	Low________	High________

ATTACHMENT 4

Dinophilus gyrociliatus REPRODUCTION DATA SHEET

Test Sample______________________________Project No.______________
Burn No.________________Date Start__________Finish______________
Concentration of Adjusted Sample________________________Initials________
Comments____
____

		3-Day		4-Day		5-Day		6-Day			7-Day		
Conc.	Rep.	E	EM	E	EM	E	EM	E	EM	L	E	EM	L
CONTROL	A												
	B												
	C												
	D												
	E												
	A												
	B												
	C												
	D												
	E												
	A												
	B												
	C												
	D												
	E												
	A												
	B												
	C												
	D												
	E												

APPENDIX B

DIOXIN/FURAN LABORATORY PROCEDURES

AND

MASS SPECTROMETRIC IDENTIFICATION AND MEASUREMENT OF PCBS AND CHLOROBENZENES AS ISOMER GROUPS

TABLE OF CONTENTS

Page

Dioxin and Furan Analysis.. B-1

- Chain of Custody/Log-In.. B-1
- Glassware Marking and Tracking.. B-3
- Glassware Preparation and Cleaning.. B-3
- Basic Alumina Preparation.. B-4
- Basic Alumina Chromatography.. B-5
- Macrocolumn Basic Alumina Chromatography.. B-6
- Silica Gel Preparation.. B-6
- Multilayer Silica Gel Chromatography.. B-7
- Florisil Preparation.. B-8
- Florisil Chromatography.. B-8
- Soxhlet Extraction.. B-9
- Separatory Funnel Extraction.. B-10
- Separatory Funnel Washes.. B-11

Mass Spectrometric Identification and Measurement of PCBs and Chlorobenzenes as Isomer Groups.. B-12

1. Scope and Application.. B-12
2. Summary of Method.. B-13
3. Definitions.. B-15
4. Interferences.. B-15
5. Safety.. B-15
6. Apparatus and Equipment.. B-16
   - 6.1 Computerized GC/MS.. B-16
   - 6.2 GC Column.. B-16
7. Reagents and Consumable Materials.. B-17
8. Calibration.. B-18
9. Quality Control.. B-19
10. Procedures.. B-19
    - 10.1 GC/MS Analysis.. B-19

References.. B-20

STANDARD OPERATING PROCEDURES FOR DIOXIN AND FURAN ANALYSIS

CHAIN OF CUSTODY/LOG-IN

Establishment of sample chain of custody is extremely important for all samples submitted for PCDD/PCDF analyses. Due to the potential impact of the analytical results, such as health concerns or termination of an industrial process, it is mandatory to maintain a record documenting the history of each sample which enters the PCDD/PCDF facility. Chain of Custody procedures enable the sponsor to trace the course of the analysis from shipment, to receipt, to analysis, to the reporting of data and/or return of the samples.

All samples are received either from in-house sampling teams or through the Shipping and Receiving Department. For in-house samples, the sampling team should notify the analysis personnel that samples have been taken and are ready for PCDD/PCDF analysis. The sampling team should then deliver the samples to the analyst in the extraction laboratory. For samples received from outside Battelle, samples will be received in the Shipping and Receiving Department. The personnel in this department have been instructed not to open any package and to isolate all packages suspected of being damaged. Upon arrival of a sample shipment, the extraction personnel will be notified and the samples transported directly to the extraction laboratory. It is imperative that all receiving documents be signed in shipping to acknowledge receipt before the samples are taken to the extraction laboratory. A copy of the shipping bill should be made, if available, and retained as a record of receipt.

Sample shipments may be received in several types of containers including chest coolers, reinforced cardboard boxes, and various assorted paper packages. Because of the potential hazard involved in shipping samples suspected of containing PCDD/PCDFs, every effort should be made to instruct the sponsor in the proper shipping procedures.

When opening shipping containers, assume the sample containers may be broken and take appropriate safety precautions. Gloves, lab apron, and safety glasses should be worn. The shipping containers should be opened in a fume hood if possible. If not, the sample containers must be transferred directly to the hood upon opening the shipping containers.

Once the samples have been removed from the shipping containers, locate the chain of custody sheets or packing list. This will be a record of what was sent by the sponsor and the laboratory's indication of what should have been received. Sign, date, and record the time of receipt on the custody sheets. Check each sample against the chain of custody sheet to insure that all samples were received. If any are missing or there are more than listed, note this on the custody sheets. Examine each sample container for damages. If present, note this on the custody sheets and place the sample in a suitable container.

The log-in process is designed to assign a unique number to each sample that will follow the sample through the entire analysis process. The number given is the BCL number and consists of the Book number - Page number - Line number of the laboratory record book where the sample is described. This description should include any number/letter designation given by the client, a physical description of the sample, and any other information pertaining to the collection, weight, surface area, damage, etc. of the sample. Following the listing of all the samples in the record book a brief description of the sample shipment receipt should be given. This should include carrier, time and day of receipt and by whom, type of container, shipper if different from client, and place of storage until analysis begins. Also, copies of the chain of custody sheets (packing slips), shipping bill (if available), and any accompanying letters should be taped onto a separate page in the lab record book. The originals of these documents along with a photocopy of the log-in page of the lab record book should be given to the principal investigator for the case file records.

Once the BCL numbers have been recorded in the lab record book, a label displaying the BCL number and client name should be attached to each sample container. The samples should be stored in their shipping containers (if possible) in the dioxin lab storage area.

GLASSWARE MARKING AND TRACKING

To assure proper QA/QC in regards to glassware, a system has been set up to "track" glassware usage and thus limit the possibility of cross contamination and carryover. All glassware will be etched with a code to identify each individual piece used during the extraction process. The code description and a list of the glassware will be recorded in the QA lab book 38701. When a sample is extracted, the identity of each piece of glassware used will be recorded in the extraction laboratory record book. After the extraction, the glassware will be cleaned according to the specified procedure (see Glassware Preparation and Cleaning) and , ideally, not used again until the analysis of that sample has been completed. If high levels are indicated or suspected, glassware blanks should be run and the cleaning procedure repeated. There will be three designations of glassware: Trace, High, and Normal level. The designations refer to the expected levels of contaminants in the sample and can be separated by the general levels of trace <100 ppt, normal 100 ppt, >100 ppb, and high >100 ppb. Each piece of glassware is marked with a letter to indicate the type of glassware followed by a sequential number. If the piece belongs to a special "set" of glassware, the code will be preceded by either a T for trace or an H for high level. Normal level glassware will have no special designation.

Glassware blanks will be run when indicated and periodically to ensure adequate QA/QC. The blanks will consist of methylene chloride "extractions" of the glassware followed by concentration and addition of internal standards. The "extracts" will be analyzed directly without column chromatography.

GLASSWARE PREPARATION AND CLEANING

Proper cleaning and glassware preparation is essential for trace level quantitative analysis, thus extensive measures must be taken to ensure that no interferences are introduced into the sample via the glassware. The following materials and procedures should be used to clean glassware. Solvent rinses are performed with distilled-in-glass quality acetone, methylene chloride, and toluene. Soap and water washes should be performed with hot tap water and Alconox detergent and rinsed with distilled water. Oxidizing acid bath consists of reagent grade concentrated sulfuric acid and No-Chromix cleaning solution prepared according to the manufacturer's instructions. The muffle furnace should be operated in eight hour cycles at a temperature of 450°C.

Samples should be removed from the glassware and discarded (see waste disposal). The apparatus should then be rinsed generously with acetone and methylene chloride. If the glassware appears discolored or is suspected of having inorganic residues, it should be placed in the oxidizing acid bath for at least four hours (see safety section before using acid bath). After removing the glassware, rinse with tap water and wash with detergent. Rinse with distilled water and allow to air dry. Rinse the glassware with acetone followed by a generous rinse with toluene. The toluene is allowed to evaporate and the glassware is muffled at 450°C for an eight hour cycle. It is advisable to repeat this procedure just prior to use to insure no contamination.

All extraction and general laboratory glassware should be cleaned as above. Soxhlet condensers may only be solvent rinsed with toluene between samples but should be thoroughly cleaned in between programs or after suspected high level samples. Fritted glass extraction thimbles may be soaked in toluene for several hours to remove trapped organics in the fritted glass. Separatory funnels should be cleaned as above and rinsed with solvent immediately before use. Sample vials and jars should be cleaned as described before use and sealed with Teflon lined caps. The chromatographic solvent reservoirs and concentration cows should be periodically disassembled and cleaned as described.

BASIC ALUMINA PREPARATION

The basic alumina (chromatographic grade aluminum oxide as 100/200 mesh BioRad Basic Aluminum AG-10, BioRad Laboratories, Richmond, CA) is activated by placing the alumina in a Pyrex glass tube furnace and increasing the temperature stepwise to 300°C (50°/5 min intervals) under nitrogen purge.

This temperature is maintained for 60 minutes. The glass tube is then removed and cooled to ambient temperature. Nitrogen purging is discontinued and the alumina is washed with 350 mL of methylene chloride. The methylene chloride saturated absorbent is returned to the furnace and heated to 50°C under nitrogen purge. This is continued for 25 minutes, after which the temperature is gradually increased to 300°C at 5°/min and maintained for 90 minutes. The alumina is then transferred to sample jars and stored in an oven at 110°C until it is used.

BASIC ALUMINA CHROMATOGRAPHY

The absorbent should be prepared as described previously. Prepare the chromatography column by removing the top two inches of a disposable 5 mL pipet (mm OD x mm length) and placing a plug of soxhlet extracted glass wool in the bottom. Pack the column with 1.0 g basic alumina and tap the absorbent down gently. Prewash the column with 10 mL of hexane and discard the eluate. Quantitatively transfer the extract, by means of a disposable Pasteur pipet, to the top of the column. When the solvent level reaches the top of the absorbent, rinse the extract container with 3 x 5 mL hexane and add this to the top of the column. Elute the column with 5 mL 3 percent methylene chloride in hexane and discard the eluate. Elute the column with 20 mL of 50 percent methylene chloride in hexane and collect the eluate in a 22 mL concentrator tube while evaporating under a stream of nitrogen. Concentrate the extract to near dryness under purified nitrogen then continue the concentration till dryness in a tube heater (temperature should be less than 50°C) with a very gentle stream of nitrogen. Rinse the tube with 3 x 1 mL portions of methylene chloride, concentrating to near dryness between each rinse. After the last rinse, concentrate the extract to dryness as above, the reconstitute the extract in an appropriate volume of n-decane. Store the extract at 0°C and darkness until analysis.

MACROCOLUMN BASIC ALUMINA CHROMATOGRAPHY

Prepare the basic alumina as described previously. The column consists of a custom made tapered column into which is placed a plug of soxhlet extracted glass wool. The column is then packed with 5-6 g of basic alumina which should be lightly tapped down. The column should be filled to a level just above the first taper. Prewash the column with 25 mL of hexane and discard the eluate. When the solvent level reaches the top of the absorbent bed, transfer the extract to the top of the column by means of a disposable Pasteur pipet. Rinse the extract container with 3 x 2 mL hexane rinses and transfer these to the top of the column. Elute the column with 15 mL hexane once the solvent layer has reached the top of the absorbent bed. Then elute with 25 mL 3 percent methylene chloride in hexane or 25 mL 50 percent carbon tetrachloride in hexane. Either solvent is effective. Do not add the second solvent until the level of the hexane has reached the top of the absorbent bed. You should save these fractions if there is concern over recoveries or overloading the column. Elute the column with 22 mL 50 percent methylene chloride in hexane and collect the eluate in a concentrator tube under a gentle stream of nitrogen. The column may be "stripped" with 25 mL of methylene chloride. This will remove most compounds left on the column. If this is the last step in the clean up procedure concentrate and exchange the sample into n-decane as described in the 1 g basic alumina chromatography procedure. Otherwise, concentrate the sample under purified nitrogen to approximately 2 mL.

SILICA GEL PREPARATION

Silica gel (chromatographic grade silicic acid as 100/200 mesh BioSil A, BioRad Laboratories, Richmond, CA) is activated by heating in a Pyrex glass tube furnace under purified nitrogen purge at a temperature of 180°C for one hour. The glass tube is then removed from the furnace and cooled to ambient temperature while maintaining the nitrogen flow. Nitrogen is discontinued and the silica washed with 350 mL aliquots of methanol and methylene chloride consecutively. The methylene chloride saturated absorbent is returned to the furnace and heated to 50°C with nitrogen purge. After 20 minutes, the temperature is raised to 180°C in a stepwise manner and maintained for 90 minutes. The dry silica is cooled to ambient temperature and transferred to a fritted glass extraction thimble where it is soxhlet extracted for 12 hours with methylene chloride. The silica is then dried under nitrogen and heated to 180°C for one hour. The silica is cooled and transferred to sample jars and stored in a desiccator over phosphorous pentoxide.

MULTILAYER SILICA GEL CHROMATOGRAPHY

Absorbents should be prepared daily as needed.

44 percent Concentrated Sulfuric Acid on Silica -- The silica support is prepared as described previously. Approximately 35 g of silica is placed in an 8 oz glass jar to which is added 15 mL concentrated sulfuric acid. The silica is manually shaken until no clumping can be observed. The silica is then stored over phosphorous pentoxide until used.

33 percent 1N Sodium Hydroxide on Silica -- The silica support is prepared as described previously. Approximately 35 g of silica is placed in an 8 oz glass jar to which is added 17 mL 1N aqueous sodium hydroxide. The material is manually shaken until no clumping can be observed. The silica is the stored tightly sealed in the glass jar.

The column is prepared by removing the top two inches of a 25 mL disposable pipet (mm OD x mm length) and placing a plug of soxhlet extracted glass wool in the bottom. The column is then successively packed with 1.0 g silica gel, 2.0 g 33 percent (w/w) 1N NaOH on silica, 1.0 g silica gel, 4.0 g 44 percent (w/w) concentrated sulfuric acid on silica, and 2.0 g silica. Tap the absorbent down after each layer is added. It is sometimes necessary with aqueous samples to place a 1 cm layer of purified sodium sulfate on top of the last layer of silica. This will remove any residual water in the organic solvent. The column is then washed with 30 mL hexane which is discarded. The extract is then quantitatively transferred to the column, via disposable pipet, when the solvent level reaches the top of the absorbent bed. The extract container is the washed with 3 x 5 mL hexane rinses and this is added to the top of the column. The extract may be in any number of solvents including hexane, methylene chloride, 1:1 hexane in benzene, or 1:1 hexane in toluene. The column is then eluted with an appropriate volume of hexane to give a total eluate of 90 mL. The entire eluate is collected and concentrated under purified nitrogen. With all extract solvents except hexane, the eluate must be concentrated to very near dryness then diluted with 2 mL hexane. This is done by concentrating the effluent to approximately 2 mL with nitrogen then placing the extractcontainer in a tube heater (temperature should be less than 50°C) and passing a very gentle stream of nitrogen over the extract until it is nearly dry. Do not let the nitrogen blow on a dry sample.

FLORISIL PREPARATION

The florisil (chromatographic grade Florisil as 60/80 mesh purchased from Floridin Co) is activated by placing it into a Pyrex glass tube furnace and washing with 350 mL of methylene chloride. The methylene chloride saturated absorbent is placed in the furnace and heated to 50°C under nitrogen purge for 25 minutes. The temperature is then increased to 130°C for at least 12 hours. The florisil is then cooled to ambient temperature and nitrogen purge discontinued. The florisil is stored in an oven at 130°C until it is used.

FLORISIL CHROMATOGRAPHY

Prepare the Florisil as described previously. The column consists of a custom made tapered column into which is placed a plug of soxhlet extracted glass wool. The column is then packed with 5 g of Florisil which should be lightly tapped down. The column should be filled to a level just above the first taper. Prewash the column with 25 mL of hexane and discard the eluate. When the solvent level reaches the top of the absorbent bed, transfer the extract to the top of the column by means of a disposable Pasteur pipet. Rinse the extract container with 3 x 2 mL hexane rinses and transfer these to the top of the column. Elute the column with 15 mL of hexane once the solvent layer has reached the top of the absorbent bed. Then elute with

25 mL of 6 percent ethyl ether in hexane followed by 75 mL of 25 percent hexane in methylene chloride. Collect the 25 percent hexane in methylene chloride fraction and concentrate the sample under purified nitrogen to approximately 2 mL.

SOXHLET EXTRACTION

Place 5-15 g of precleaned silica gel into the fritted glass thimble. The amount will depend on the type of sample. For wipes and filters only a small amount is necessary while a larger portion will be needed for very fine particulate samples. Also place a plug of soxhlet extracted glass wool into the thimble. Place the thimble into the extractor body, fill the boiling flask with the appropriate volume of benzene, assemble the apparatus, and reflux for a minimum of four hours immediately before beginning the extraction. At the end of this pre-extraction period, discard the solvent and rinse the apparatus with additional solvent. Allow the thimble to air dry before weighing the sample into it. If no weight is necessary such as a wipe sample, the drying is not necessary. Weigh the sample, by difference, into the thimble to three significant figures. For wipe and filter samples, place the sample into the thimble using tweezers. For small samples, distribute the sample as evenly as possible over theentire silica bed. For carbonaceous material the sample bed should be as thin as possible to expose as much surface area as possible to the extracting solvent. After the sample is in the thimble, spike it with the appropriate volume of internal standard solution, distributing the spike evenly over the entire sample. It may be necessary to "wet" the sample with a small amount of solvent before spiking in the case of carbonaceous material to deactivate some of the active sites thus avoiding complete loss of the internal standard to irreversible absorption. Place the thimble into the extractor body and rinse the sample container into the thimble with solvent if the entire sample was used. Place the plug of glass wool into the thimble directly on top of the sample. Fill the boiling flask with the appropriate volume of solvent and assemble the apparatus. During assembly, seal each ground glass joint with a small amount of solvent and wrap the joints with Teflon tape. A steady flow of water must be maintained through the condensers throughout the extraction. Extract the sample at maximum reflux rate, approximately 20 mL/min ,for a minimum of 18 hours. For ash and carbonaceous material it may be necessary to extend the extraction period to at least 24 hours. See safety precautions for overnight reactions. At the conclusion of the extraction period, cool the apparatus and drain the solvent into the boiling flask. This is done repeatedly until all of the solvent has been drained from the thimble. Remove the extractor and fit the boiling flask with a three stage Snyder column. Reduce the volume to approximately 5 mL by atmospheric distillation. Rinse the column and the flask with an equal volume of hexane giving a 1:1 hexane in benzene extract. The extract should be put through the clean up procedure immediately. Should it be necessary to store the extract until clean up, seal the flask with a ground glass stopper and cover the flask with aluminum foil.

SEPARATORY FUNNEL EXTRACTION

The sample should be shaken vigorously before the aliquot is removed so that any sediment, if present, will be in suspension. If sediment or particulate matter is present, shake the sample and pour an aliquot into a graduated cylinder by passing it through Whatman 541, 24cm filter paper. The average sample volume for aqueous samples should be 1000 mL. If the entire sample is used, rinse the container with methylene chloride and transfer this directly to the separatory funnel. If it is necessary to filter the sample, save the filter paper as it may be necessary to extract it separately. Record the volume of the sample and transfer the sample to an appropriate sized separatory funnel. Spike the sample with the appropriate volume of internal standard by means of an Eppendorf pipet. Dissolve the spike in 1-2 mL of acetone to increase the solubility of the spike solvent in water and transfer this solution to the sample with a few hundred µL of additional acetone. Allow the spike to equilibrate for several minutes before beginning the extraction. The sample is extracted with three 60 mL portions of methylene chloride. During each extraction, the sample should be shaken for approximately one minute, venting the funnel frequently. Too vigorous shaking may produce emulsions in some samples. If an emulsion does form, a few mL of a saturated Na_2CO_3 solution may be added and the funnel gently swirled until the emulsion breaks. The phases should be allowed to separate before each extract portion is drawn off. The portions should be collected in a Kuderna-Danish apparatus and concentrated to approximately 2 mL using a three stage Snyder column in a water bath. The apparatus should be rinsed with hexane, the KD flask and Snyder column removed, and the volume reduced to 2 mL using a micro Snyder column in a water bath. The hexane extract should then be passed through a microcolumn containing approximately 10 g of Na_2SO_4 and eluting it with an additional 30 mL of hexane. This should be collected and concentrated to approximately 2 mL by purified nitrogen. KD sample tubes with ground glass stoppers should be used to store the samples before clean up and the samples should be protected from continuous exposure to light.

SEPARATORY FUNNEL WASHES

Transfer the sample extract in organic solvent to an appropriate sized separatory funnel. The extract should be in hexane or hexane-benzene and the extract container should be rinsed with 3 x 5 mL hexane and the rinses transferred into the separatory funnel. The separatory funnel should be of sufficient size so that the extract volume is only half the capacity of the funnel. The sample is then liquid-liquid extracted with 10 mL portions of concentrated sulfuric acid. The acid should be added dropwise to prevent a violent reaction. If the extract is extremely dirty, the first "wash" should be made with 10 mL of 1:1 concentrated sulfuric acid/distilled water. This will prevent polymerization and clumping that may occur in extremely dirty samples when pure concentrated sulfuric acid is used. The remaining rinses should then be with concentrated sulfuric acid. The funnels should be shaken gently at first with frequent venting. The extract should be allowed to stand for a few minutes to allow additional contact time between the acid and organic layers. This should be repeated for several series of shakings. After the final shake, the two phases should be allowed to separate for several minutes before removing the acid layer. The acid washes are repeated until the acid layer is only lightly colored or to a maximum of 5 washes. If more than one wash is necessary, the acid layers should be back extracted to prevent loss of the analytes. Each acid fraction, after the extract wash, is collected in another 250 mL separatory funnel. When all of the fractions are composited, they are back-extracted with 20 mL of hexane. The acid is then discarded and the hexane is added to the original sample extract. If only one sample wash is necessary, the acid fraction is discarded without back-extraction. The organic sample extract is then washed with 20 mL of distilled water to remove any residual acid. This is also discarded. If necessary, a rinse with 1M aqueous sodium hydroxide can be performed. The rinse should consist of a 10 mL aliquot of 1M NaOH and is discarded after the wash.. If any darkening occurs, repeat the wash. The extract is then washed with another 20 mL portion of distilled water. The extract can then be passed through a microcolumn containing 5 g of anhydrous sodium sulfate and eluting the column with 30 mL additional hexane. The entire elute is then collected and concentrated to approximately 2 mL by either a Kuderna-Danish apparatus or under nitrogen.

MASS SPECTROMETRIC IDENTIFICATION AND MEASUREMENT OF PCBS AND CHLOROBENZENES AS ISOMER GROUPS

1. SCOPE AND APPLICATION

1.1. This method provides procedures for identification and measurement of polychlorinated biphenyls (PCBs) and polychlorinated benzenes (CBs) as isomer groups (i.e., by level of chlorination). Because PCBs are identified and measured as isomer groups, each non-specific CASRN for each level of chlorination is used to describe method analytes.

Monochlorobiphenyls	C12H9Cl	27323-18-8
Dichlorobiphenyls	C12H8Cl2	25512-42-9
Trichlorobiphenyls	C12H7Cl3	25323-68-6
Tetrachlorobiphenyls	C12H6Cl4	26914-33-0
Pentachlorobiphenyls	C12H5Cl5	25429-29-2
Hexachlorobiphenyls	C12H4Cl6	26601-64-9
Heptachlorobiphenyls	C12H3Cl7	28655-71-2
Octachlorobiphenyls	C12H2Cl8	31472-83-0
Nonachlorobiphenyls	C12HCl9	53742-07-7
Decachlorobiphenyl	C12Cl10	2051-24-3
Chlorobenzene	C6H5Cl	108-90-7
1,2-Dichlorobenzene	C6H4Cl2	95-50-1

1,3-Dichlorobenzene	$C_6H_4Cl_2$	541-73-1
1,4-Dichlorobenzene	$C_6H_4Cl_2$	106-46-7
1,2,3-Trichlorobenzene	$C_6H_3Cl_3$	87-61-6
1,2,4-Trichlorobenzene	$C_6H_3Cl_3$	120-82-1
1,3,5-Trichlorobenzene	$C_6H_3Cl_3$	108-70-3
1,2,3,4-Tetrachlorobenzene	$C_6H_2Cl_4$	634-66-2
1,2,3,5-Tetrachlorobenzene	$C_6H_2Cl_4$	634-90-2
1,2,4,5-Tetrachlorobenzene	$C_6H_2Cl_4$	95-94-3
Pentachlorobenzene	C_6HCl_5	608-93-5
Hexachlorobenzene	C_6Cl_6	118-74-1

1.2 Method Detection Limits (MDLs) (1) have not been statistically determined.

2. SUMMARY OF METHOD

2.1 Sample extract components are separated with capillary column gas chromatography (GC), PCBs and CBs are identified and measured as isomer groups (i.e., by level of chlorination) with electron ionization (EI) mass spectrometry (MS). Either full-range or selected-ion-monitoring (SIM) data are acquired, depending on the concentration range of concern. An interfaced data system (DS) to control data acquisition and to store, retrieve, and manipulate mass spectral data is essential.

Retention times of PCB congeners are not an important identification criterion. Although retention times of the 209 PCB congeners tend to increase with increasing chlorine content, individual members ofisomer groups display retention times that are highly dependent on structure. For example, even with capillary columns, some PCBs containing five chlorines have retention times equivalent to those for PCBs containing four or six chlorines. An apparent singlecomponent GC peak may contain coeluting or partially coeluting PCB congeners with different levels of chlorination. Therefore, assignment of level of chlorination cannot rely on GC retention times. Instead, knowledge of the characteristic features of PCB mass spectra is used to assess sample component spectra to identify each PCB as a member of an isomer group.

Retention times of CB congeners are not an important identification criterion. The retention times of the 12 CB congeners tend to increase with increasing chlorine content. With capillary column chromatography there is no overlap of CB congeners with different levels of chlorination. However, within a given level of chlorination some of the congeners may coelute or partially coelute, making the identification and measurement of the individual congeners difficult. Therefore, identification and measurement by level of chlorination based on characteristic features of the CB mass spectra provides useful information without requiring specific identification of each congener.

The most important EI mass spectral feature is the molecular ion (M+) cluster (2). The combination of masses and known relative abundances of M+ cluster ions is used as the primary identification criterion. For each isomer group, one of the M+ cluster ions is used as the quantification ion and one is used as the confirmation ion.

Coelution of PCBs containing fewer chlorines than a given isomer group does not interfere with detection and measurement of M+ ions produced by a member of that isomer group, but coeluting congeners containing more chlorines do interfere. PCBs have characteristic (M-70)+ ion clusters that overlap or partially overlap the M+ ion cluster produced by a congener containing one or two fewer chlorines. Therefore, tentative assignment of a level of chlorination based on detection of two ions in an M+ ion cluster must be verified by the absence of pertinent higher mass ions, and observed relative abundances of isotope cluster ions must be sufficiently similar to expected relative abundances.

A concentration is measured for each isomer group; total PCB and CB concentration in each sample extract is obtained by summing their individual isomer group concentrations. Nine selected PCB congeners are used as concentration calibration standards. One PCB congener is used as the concentration calibration standard for all isomers at each level of chlorination, except that decachlorobiphenyl is used for all isomers containing nine and ten chlorines. One internal standard, chrysene-d12, is used to calibrate MS response. Six selected CB congeners are used as concentration calibration standards. One CB congener is used as the concentration calibration standard for all of the isomers at each level of chlorination. Phenanthrene-d10 is used as the internal standard to calibrate the MS response.

2.2. Special Incos software performs automated, unattended identification and measurement of PCBs. Unprocessed GC/MS data are handled without human interaction with the software operating on the dedicated minicomputer.

3. DEFINITIONS

3.1. Internal standard -- A pure compound added to a sample extract in known amounts and used to calibrate concentration measurements of other compounds that are sample components. The internal standard must be a compound that is not a sample component.

3.2. Congener number -- Throughout this method, individual PCBs are described with the number assigned by Ballschmiter and Zell (3). (This number is also used to describe PCB congeners in catalogs produced by Ultra Scientific, Hope, RI.)

4. INTERFERENCES

4.1. With MS detection of PCBs and CBs, interference can be caused by the presence of much greater quantities of other sample components that overload the capillary column; additional sample extraction preparation procedures must than be used to eliminate interference. Other chlorinated sample components that produce the same quantification and confirmation ions also may interfere, even when present in relatively small amounts. The approach and identification criteria used in this method, however, eliminate interference by most chlorinated compounds except other PCBs. With the isomer group approach, coeluting PCBs that contain the same number of chlorines are identified and measure together. Therefore, coeluting PCBs are a problem only if they contain different numbers of chlorine atoms. This interference problem is obviated by automated application of rigorous identification criteria.

5. SAFETY

5.1. The toxicity and other harmful effects of PCBs and other chemicals used in this method are not completely defined; each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized. Some PCBs have been classified as known or suspected carcinogens.

5.2. Each laboratory is responsible for maintaining awareness of safety procedures for handling chemicals used in this method. Primary standards of PCBs should be prepared in a hood by personnel wearing protective equipment, including full-length lab coat, safety glasses, and disposable gloves.

6. APPARATUS AND EQUIPMENT

6.1. Computerized GC/MS System

6.1.1. The GC must be capable of temperature programming and be equipped with all required accessories, such as syringes, gases, and a capillary column. The GC injection port must be designed for capillary columns; an on-column capillary injector system is desirable but not essential.

6.1.2. Both full range and selected ion monitoring (SIM) mass spectral data are obtained with electron ionization at a nominal electron energy of 70 eV. To ensure sufficient precision os mass spectral data, the required MS scan rate must allow acquisition of at least five full-range mass spectra or five data points for each monitored ion while a sample component elutes from the GC. The MS must produce a mass spectrum meeting specified criteria when 50 ng of decafluorotriphenylphosphine (DFTPP) is introduced through the GC inlet.

6.1.3. An interfaced Incos data system (DS) is required to acquire, store, reduce, and output mass spectral data. The DS must be capable of searching a data file for specific ions and plotting ion abundances versus time or spectrum number to produce selected ion current profiles (SICPs) and extracted ion current profiles (EICPs). Also required is the capability to obtain chromatographic peak areas between specified times or spectrum numbers in SICPs or EICPs. The Ds must be equipped with a SIM program capable of acquiring data for at least seven sets of ions (limited-mass ranges) consisting of a total of ≤33 per set, and the DS must allow automated and rapid changes of the set of ions being monitored. The times spent monitoring ions during sample analysis must be the same as the times used when concentration calibration solutions were analyzed. Total data acquisition time per cycle must not exceed 1.5 seconds.

6.2. **GC Column -- A 30 m X 0.25 mm ID fused silica capillary column** coated with a 0.25 um thick film of DB-5.

Operating conditions known to produce acceptable results are shown in Table B-1. Retention times have been reported (4) for all 209 PCB congeners with a column coated with SE-54, which provides retention times essentially identical to those obtained with DB-5.

7. REAGENTS AND CONSUMABLE MATERIALS

7.1. MS Calibration Check Solution -- Prepare a 50 ng/µL solution of decafluorotriphenylphosphine (DFTPP) in an appropriate solvent.

7.2. Calibration Solutions -- Six hexane solutions containing each of the nine individual PCB calibration compounds, the internal standard (chrysene-d12), and three additional PCB congeners that are used to establish GC conditions for SIM data acquisition. One isomer at each level of chlorination is used as the concentration calibration standard for all other isomers at that level of chlorination, except that decachlorobiphenyl (Cl10) is used for both Cl9 and Cl10 isomer groups. Three solutions are used to calibrate full-range data, and three are used to calibrate SIM data.

One solution contains each calibration congener at a concentration near but greater than the anticipated MDL for that congener. Detector response decreases with increasing level of chlorination, varying by a factor of approximately six from Cl1 to Cl10 congeners. Therefore, congener concentrations in calibration solutions increase with level of chlorination. One solution contains each calibration congener at the concentration anticipated to be approximately the highest concentration to allow injection of 1-µL or 2-µL aliquots that do not saturate the MS detector. The third solution contains each congener at a concentration between the highest and lowest concentrations. Because of the difference in sensitivity of with SIM and full-range data acquisition, concentrations of calibration solutions depend on the type of data to be acquired. Concentration calibration solutions and information about their components and concentrations will be provided.

The nine PCB congeners listed in Table B-2 are used as concentration calibration compounds. Concentrations of calibration solutions are given in Table B-3. Two concentration calibration congeners also serve as retention time congeners; the first eluting Cl1-PCB indicates the time when data acquisition must have been initiated for Ion Set No. 1, and the Cl10-PCB indicates when all PCBs have eluted. Three additional PCb congeners (No. 104, a Cl5-PCB, No. 77, a Cl4-PCB, and No. 208, a Cl9-PCB) are used to establish retention times necessary to initiate data acquisition with Ion Sets No. 2-4.

Retention times of those congeners relative to chrysene-d12 are about 0.80 for No. 104, 0.90 for No. 77, and 1.07 for No. 208 (5).

8. CALIBRATION

8.1 Routine Calibration -- To be performed at the beginning of each 8-hour period during which PCB analyses are to be performed.

8.1.1. Calibrate and tune the MS with standards and procedures prescribed by the manufacturer.

8.1.2. Analyze a 1-µL aliquot of the DFTPP solution and ensure that all criteria in Table B-3 are met.

8.1.3. Inject a 1-µL or 2-µL aliquot of the medium level concentration calibration/performance check solution and analyze.

8.1.4. Determine and document acceptable performance with the following criteria:

8.1.4.1. GC separation -- Baseline separation of PCB congener No. 87 from congeners No. 154 and No. 77, which may coelute.

8.1.4.2. MS sensitivity -- Signal/noise ratio of ≥ 5 for m/z 499 of PCB congener No. 209, Cl_{10}-PCB.

8.1.4.3. MS resolution -- Baseline resolution of m/z 430 and m/z 431 for PCB congener No. 200, Cl_8-PCB.

8.1.4.4. Determine that the area measured for m/z 240 for chrysene-d12 has not varied more than 15 percent from the area measured in the most recent previous analysis of a sample extract or calibration solution.

8.1.4.5. If SIM data are being acquired, check the reproducibility of absolute retention times of congeners No. 77, No. 104, and No. 208; retention times should not vary more than ± 5 seconds from those observed during the most recent calibration.

8.1.5. Remedial actions must be taken if criteria are not met; possible remedies are:

8.1.5.1. Check and adjust GC and/or MS operating conditions.

8.1.5.2. Break off a short portion (approximately 0.33 m) of the column; check column performance by analysis of performance check solution.

8.1.5.3. Replace GC column.

8.1.5.4. Tune MS for greater or lesser resolution.

8.1.5.5. Calibrate MS mass scale.

8.1.5.6. Prepare new concentration calibration curve.

9. QUALITY CONTROL

9.1. Laboratory Reagent Blank -- Analyze a reagent blank during each 8 hour period during which analyses are to be performed. The analyst must exercise professional judgement as to whether the system is sufficiently free of contamination of proceed. A total ion current profile for each laboratory reagent blank analysis must be included in deliverables.

10. PROCEDURES

10.1. GC/MS Analysis

10.1.1. Inject a 1-µL or 2-µL aliquot of each extract into the GC operated under conditions used to produce acceptable results with the concentration calibration/performance check solution analyzed during calibration.

10.1.2. Acquire mass spectral data with either full-range data acquisition conditions or SIM conditions, as appropriate. Use the same data acquisition time and MS operating conditions previously used to determine response factors.

10.2. Identification and Measurement -- The Incos software (6) provided by EPA is used for automated identification and measurement of PCBs as isomer groups. A users' guide provides instructions for software use, and assistance will be provided by telephone, if needed.

REFERENCES

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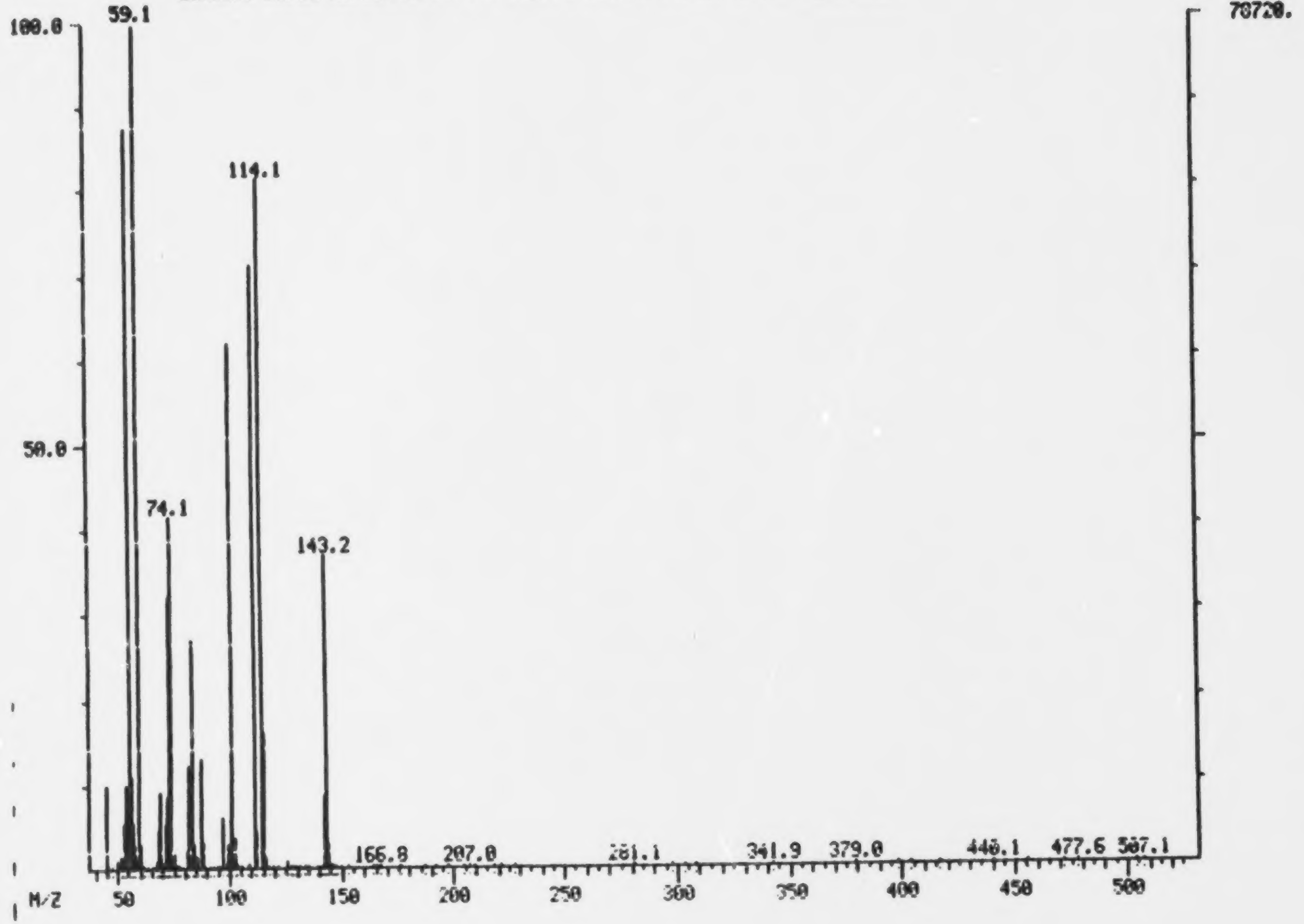
APPENDIX C

MASS SPECTRAL DATA FOR MM5 EXTRACTS AND COMPOSITE MIBAS SAMPLE

```
MASS SPECTRUM                                        DATA: 567719 #1010       BASE M/Z:  59
05/06/86 15:33:00 + 11:52                            CALI: 567610CAL32 #4     RIC:   561152.
SAMPLE: 41702-08-01 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
```



MM5 SAMPLE 1
PEAK 1
MASS SPECTRUM

```
Library Search                     Data: 567719 #1010        Base m/z:    59
05/06/86 15:33:00 + 11:52          Cali: 567610CAL32 #   4   RIC:     542719.
Sample: 41702-08-01 MMS EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

39763 spectra in LIBRARYNB searched for maximum PURITY
  295 matched at least 5 of the 16 largest peaks in the unknown

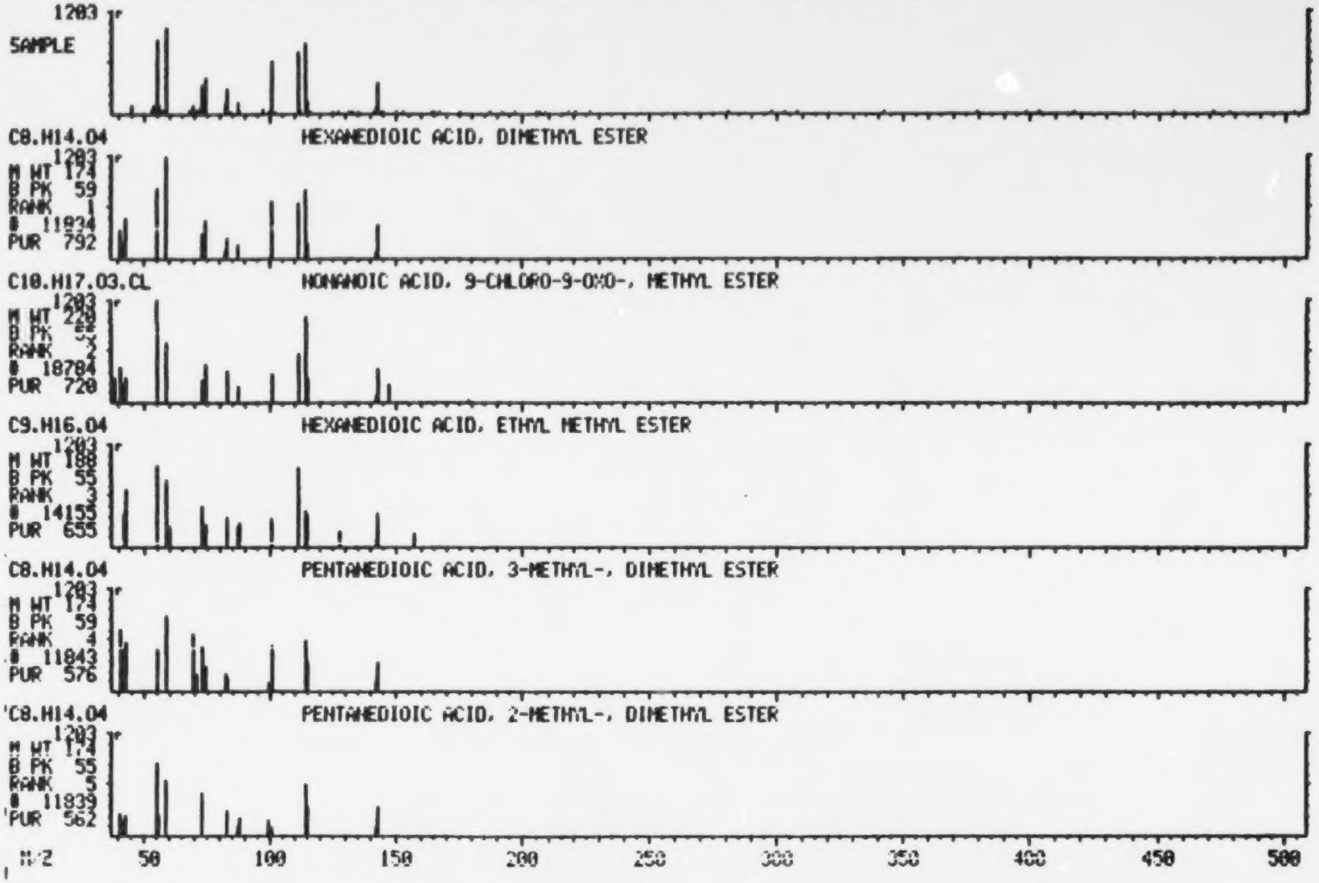
Rank In.      Name
1 11334 HEXANEDIOIC ACID, DIMETHYL ESTER
2 18784 NONANOIC ACID, 9-CHLORO-9-OXO-, METHYL ESTER
3 14155 HEXANEDIOIC ACID, ETHYL METHYL ESTER
4 11343 PENTANEDIOIC ACID, 3-METHYL-, DIMETHYL ESTER
5 11339 PENTANEDIOIC ACID, 2-METHYL-, DIMETHYL ESTER
6 11347 BUTANEDIOIC ACID, 2,2-DIMETHYL-, DIMETHYL ESTER
7  9260 HEXANEDIOIC ACID, MONOMETHYL ESTER
8 11797 PENTANEDIOIC ACID, 3-OXO-, DIMETHYL ESTER
9 14695 ARABINOFURANOSE, 2,3,5-TRI-O-METHYL-, L-

Rank  Formula                              M.Wt B.Pk   Purity    Fit   RFit
1     C8.H14.O4                             174   59      792    924    855
2     C10.H17.O3.CL                         220   55      720    857    820
3     C9.H16.O4                             188   55      655    775    786
4     C8.H14.O4                             174   59      576    789    669
5     C8.H14.O4                             174   55      562    828    603
6     C8.H14.O4                             174   73      559    767    615
7     C7.H12.O4                             160  114      544    773    648
8     C7.H10.O5                             174   69      477    681    519
9     C8.H16.O5                             192  101      460    614    517

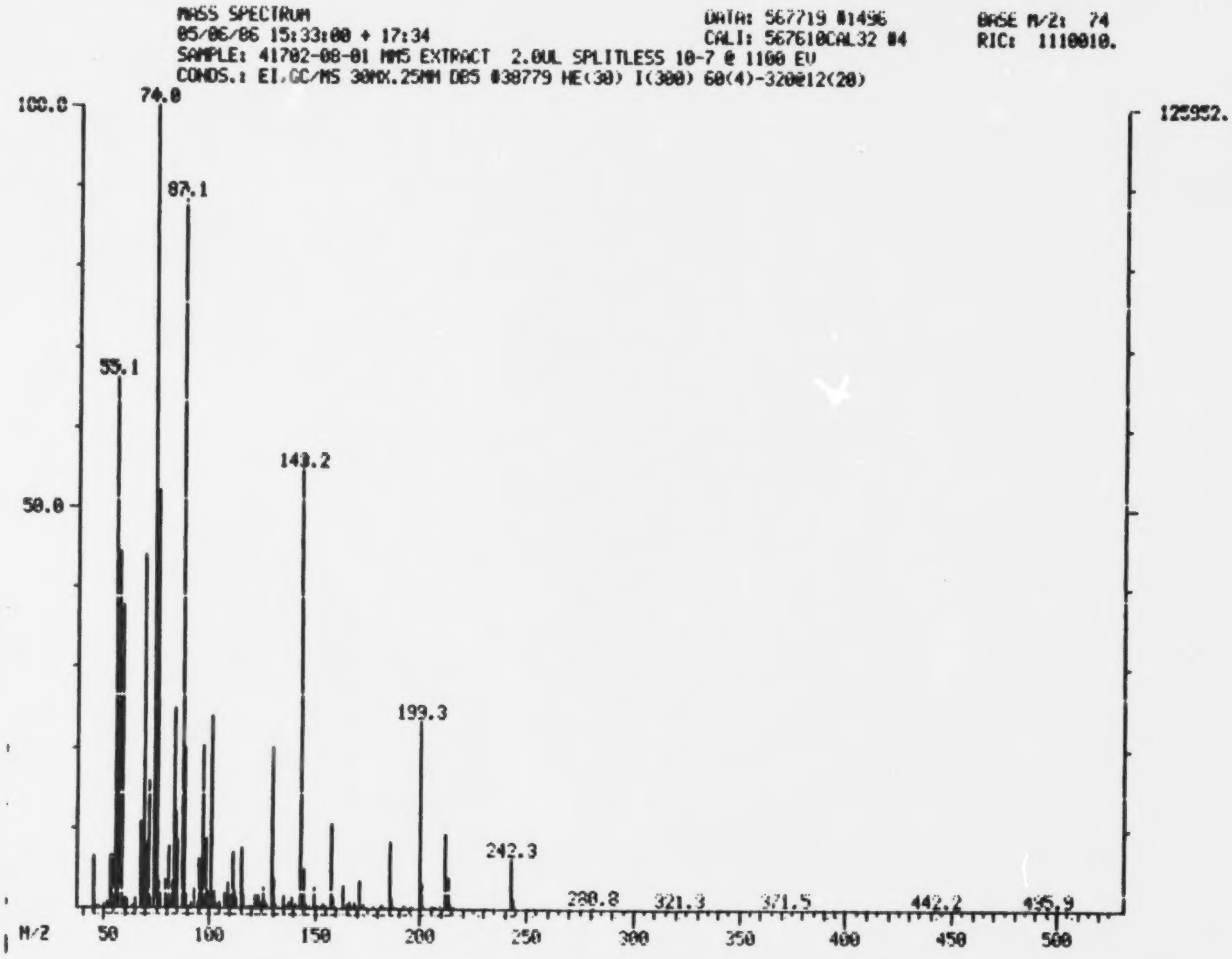
Rank      Ret.Time      B.P.Int.     VS.Par.1     VS.Par.2      C.A.S. #
1           ____          ____         0.7610       ____          627-93-0
2           ____          ____         0.6910       ____        56555-02-3
3           ____          ____         0.7610       ____        18891-13-9
4           ____          ____         0.6210       ____        19013-37-7
5           ____          ____         0.6210       ____        14035-94-0
6           ____          ____         0.3550       ____        49827-44-3
7           ____          ____         0.7590       ____          627-91-8
8           ____          ____         0.8550       ____         1830-54-2
9           ____          ____         0.4910       ____        18463-35-9
```

MMS SAMPLE 1
PEAK 1
LIBRARY SEARCH REPORT

```
LIBRARY SEARCH                                    DATA: 567719 #1010         BASE M/Z:  59
05/06/86 15:33:00 + 11:52                         CALI: 567610CAL32 #   4    RIC:    542719.
SAMPLE: 41702-08-01 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
```



MM5 SAMPLE 1
PEAK 1
COMPARISON LIBRARY MASS SPECTRA



MASS SPECTRUM
05/06/86 15:33:00 + 17:34
SAMPLE: 41702-08-01 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI.GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

DATA: 567719 #1496
CALI: 567610CAL32 #4

BASE M/Z: 74
RIC: 1110010.

MM5 SAMPLE 1
PEAK 2
MASS SPECTRUM

```
Library Search              Data: S67719 #1496          Base m/z:    74
05/06/86 15:33:00 + 17:34   Cali: S67610CAL32 #   4     RIC:    1015800.
Sample: 41702-08-01 MMS EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

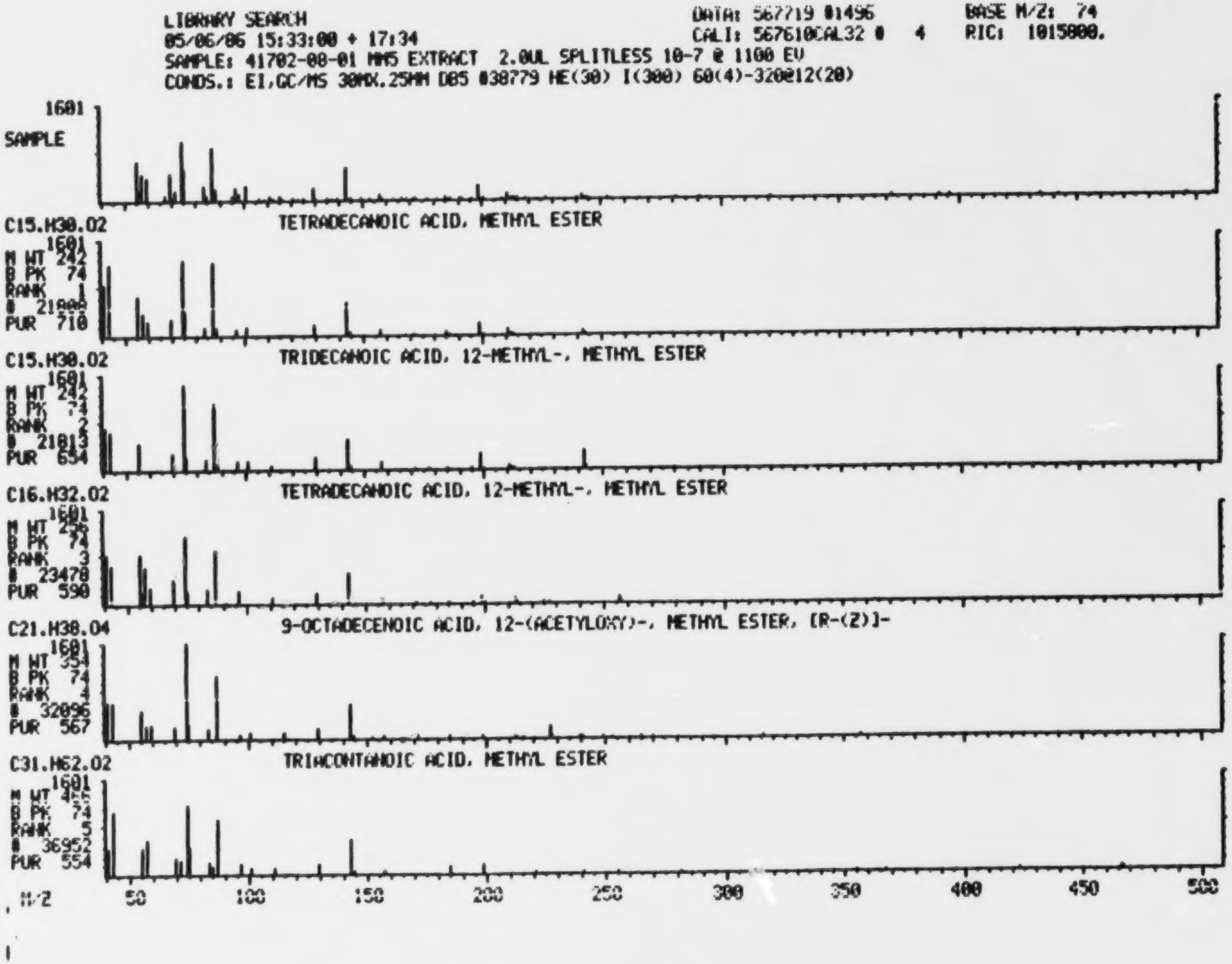
39763 spectra in LIBRARYNB searched for maximum PURITY
  457 matched at least 5 of the 16 largest peaks in the unknown

Rank In.       Name
1 21808 TETRADECANOIC ACID, METHYL ESTER
2 21813 TRIDECANOIC ACID, 12-METHYL-, METHYL ESTER
3 23478 TETRADECANOIC ACID, 12-METHYL-, METHYL ESTER
4 32096 9-OCTADECENOIC ACID, 12-(ACETYLOXY)-, METHYL ESTER, [R-(Z)]-
5 36952 TRIACONTANOIC ACID, METHYL ESTER
6 18094 UNDECANOIC ACID, 10-METHYL-, METHYL ESTER
7 15972 UNDECANOIC ACID, METHYL ESTER
8 20119 DODECANOIC ACID, 10-METHYL-, METHYL ESTER
9 27747 HEPTADECANOIC ACID, 16-METHYL-, METHYL ESTER

Rank  Formula                          M.Wt B.Pk   Purity    Fit   RFit
1     C15.H30.O2                        242   74      710    861    810
2     C15.H30.O2                        242   74      654    848    715
3     C16.H32.O2                        256   74      590    806    697
4     C21.H38.O4                        354   74      567    750    658
5     C31.H62.O2                        466   74      554    761    710
6     C13.H26.O2                        214   74      548    781    617
7     C12.H24.O2                        200   74      548    789    598
8     C14.H28.O2                        228   74      535    721    707
9     C19.H38.O2                        298   74      531    689    733

Rank    Ret.Time    B.P.Int    US.Par.1    US.Par.2      C.A.S. #
1         ----        ----      0.6440       ----        124-10-7
2         ----        ----      0.7180       ----       5129-58-8
3         ----        ----      0.6840       ----       5129-66-8
4         ----        ----      0.5950       ----        140-03-4
5         ----        ----      0.3330       ----        629-83-4
6         ----        ----      0.7210       ----       5129-56-6
7         ----        ----      0.6270       ----       1731-86-8
8         ----        ----      0.6300       ----       5129-65-7
9         ----        ----      0.6960       ----       5129-61-3
```

MMS SAMPLE 1
PEAK 3
LIBRARY SEARCH REPORT



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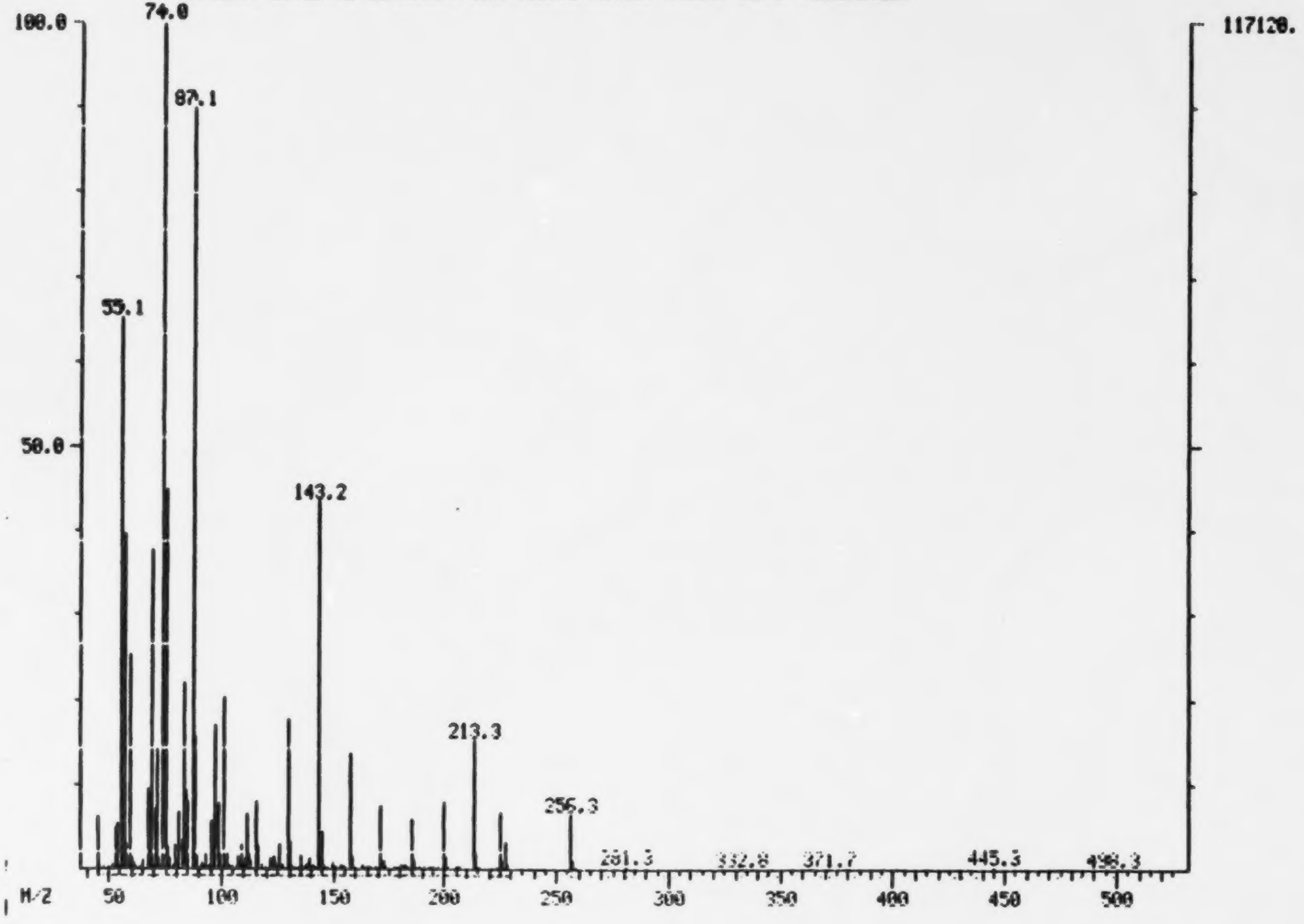
LIBRARY SEARCH                                  DATA: 567719 #1496        BASE M/Z:  74
05/06/86 15:33:00 + 17:34                       CALI: 567610CAL32 #   4   RIC:  1015800.
SAMPLE: 41702-08-01 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
```

MM5 SAMPLE 1
PEAK 2
COMPARISON LIBRARY MASS SPECTRA

MASS SPECTRUM
05/06/86 15:33:00 + 18:33
SAMPLE: 41702-08-01 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

DATA: 567719 #1580
CALI: 567610CAL32 #4

BASE M/Z: 74
RIC: 933888.



MM5 SAMPLE 1
PEAK 3
MASS SPECTRUM

```
Library Search              Data: 567719 #1580         Base m/z:    74
05/06/86 15:33:00 + 18:33   Cali: 567610CAL32 #   4    RIC:     870399.
Sample: 41702-08-01 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

39763 spectra in LIBRARYNB searched for maximum PURITY
  349 matched at least 5 of the 16 largest peaks in the unknown

Rank In.      Name
1 23479 PENTADECANOIC ACID, METHYL ESTER
2 23478 TETRADECANOIC ACID, 12-METHYL-, METHYL ESTER
3 32096 9-OCTADECENOIC ACID, 12-(ACETYLOXY)-, METHYL ESTER, [R-(Z)]-
4 21308 TETRADECANOIC ACID, METHYL ESTER
5 18094 UNDECANOIC ACID, 10-METHYL-, METHYL ESTER
6 20119 DODECANOIC ACID, 10-METHYL-, METHYL ESTER
7 23483 TETRADECANOIC ACID, 12-METHYL-, METHYL ESTER, (S)-
8 26373 HEPTADECANOIC ACID, METHYL ESTER
9 15972 UNDECANOIC ACID, METHYL ESTER

Rank   Formula                                 M.Wt B.Pk   Purity    Fit   RFit
1      C16.H32.O2                               256   74      709    876    780
2      C16.H32.O2                               256   74      684    878    751
3      C21.H38.O4                               354   74      630    836    714
4      C15.H30.O2                               242   74      591    784    693
5      C13.H26.O2                               214   74      580    833    636
6      C14.H28.O2                               228   74      556    791    612
7      C16.H32.O2                               256   74      535    917    573
8      C18.H36.O2                               284   74      534    706    622
9      C12.H24.O2                               200   74      531    774    597

Rank      Ret.Time     B.P.Int.     US.Par.1     US.Par.2       C.A.S. #
1           ___          ___         0.6410         ___          7132-64-1
2           ___          ___         0.6840         ___          5129-66-8
3           ___          ___         0.5950         ___           140-03-4
4           ___          ___         0.6440         ___           124-10-7
5           ___          ___         0.7210         ___          5129-56-6
6           ___          ___         0.6300         ___          5129-65-7
7           ___          ___         0.1360         ___         62691-05-8
8           ___          ___         0.6550         ___          1731-92-6
9           ___          ___         0.6270         ___          1731-86-8
```

MM5 SAMPLE 1
PEAK 3
LIBRARY SEARCH REPORT

```
LIBRARY SEARCH                                      DATA: 567719 #1580          BASE M/Z:  74
05/06/86 15:33:00 + 18:33                           CALI: 567610CAL32 #   4     RIC:    870399.
SAMPLE: 41702-08-01 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
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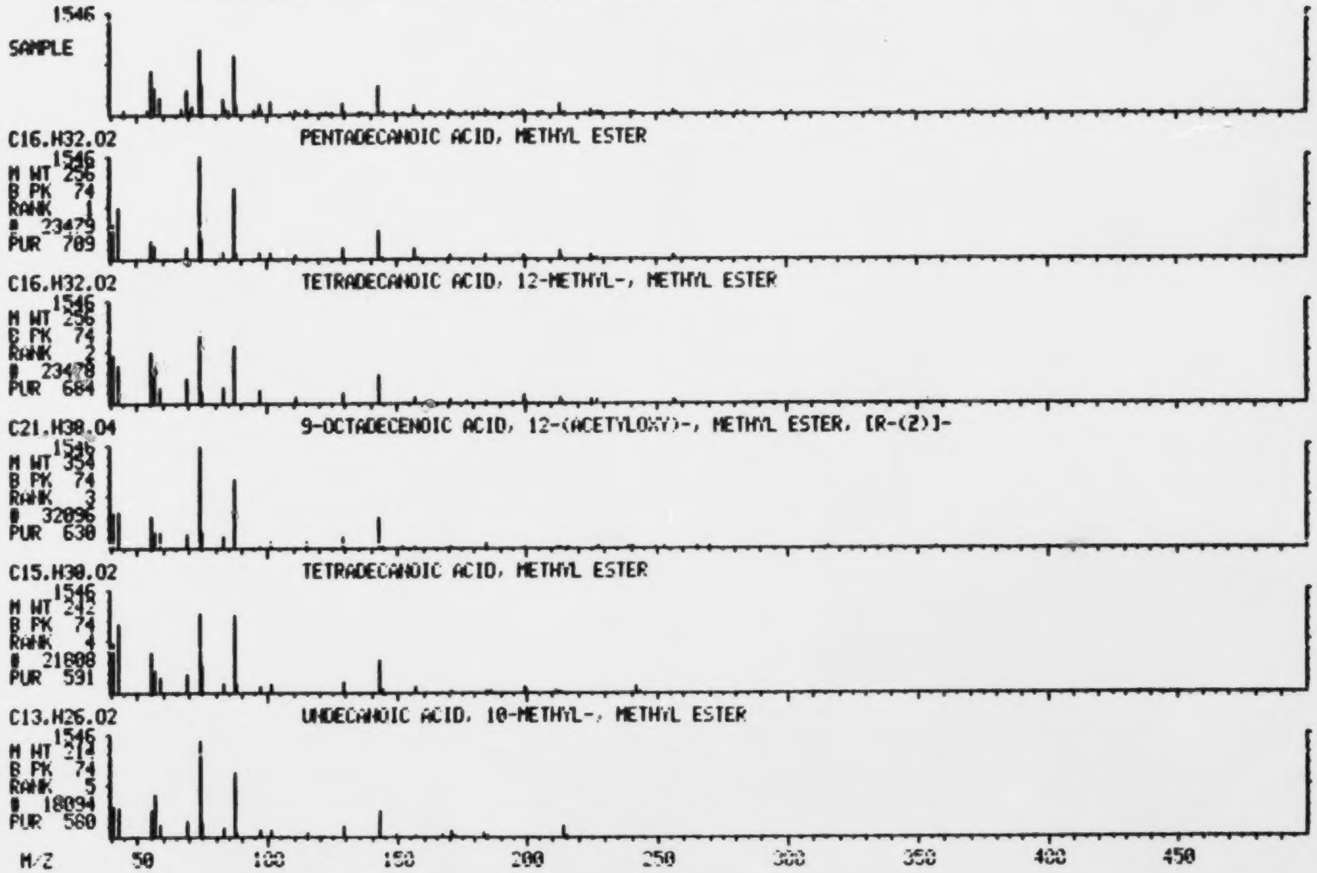
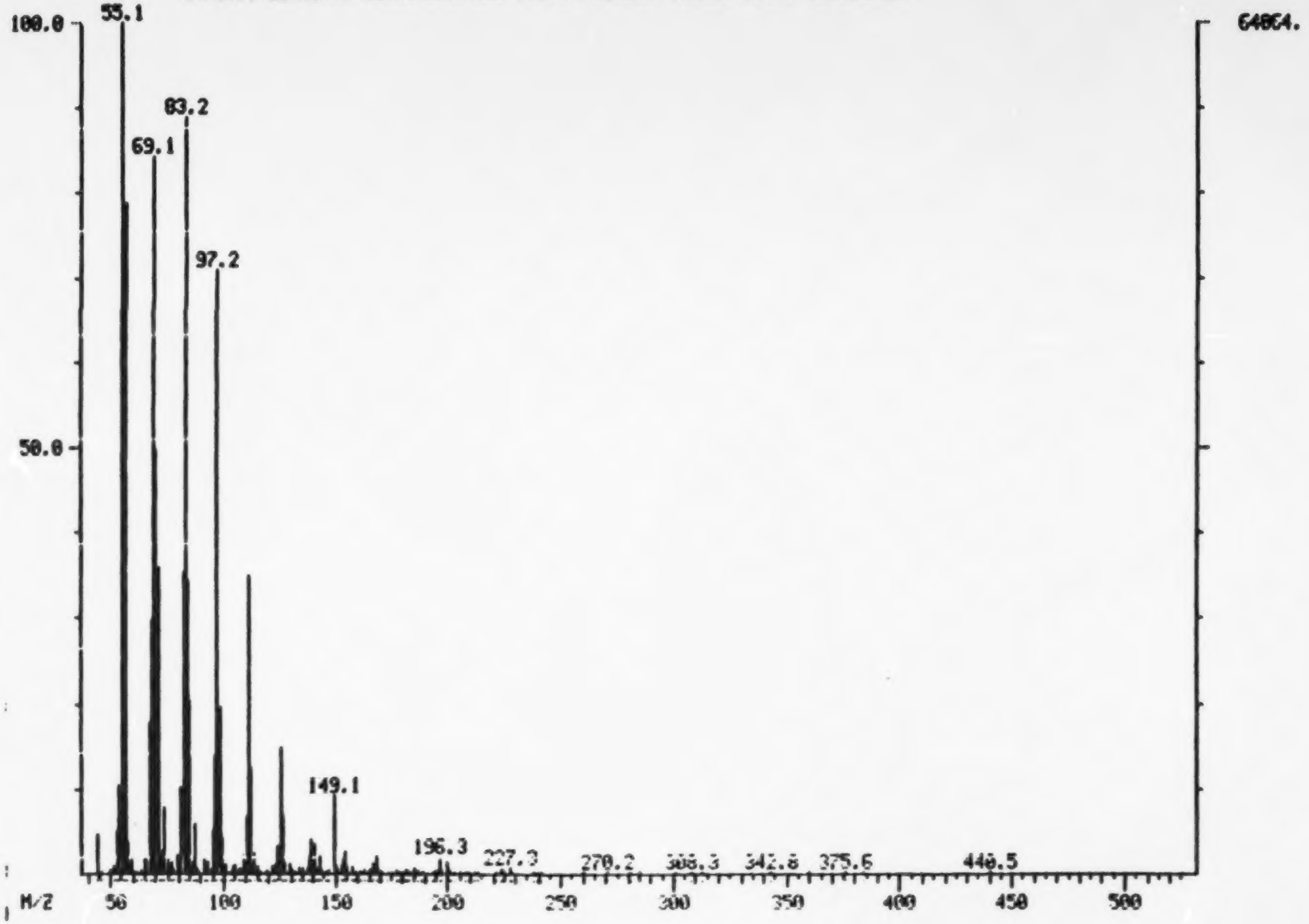


MM5 SAMPLE 1
PEAK 3
COMPARISON LIBRARY MASS SPECTRA

MASS SPECTRUM DATA: 567719 #1626 BASE M/Z: 55
05/06/86 15:33:00 + 19:06 CALI: 567610CAL32 #4 RIC: 609280.
SAMPLE: 41702-08-01 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)



MM5 SAMPLE 1
PEAK 4
MASS SPECTRUM

```
Library Search                Data:  567719 #1626          Base m/z:    55
05/06/86 15:33:00 + 19:06     Cali:  567610CAL32 #   4     RIC:      580607.
Sample: 41702-08-01 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

39763 spectra in LIBRARYNB searched for maximum PURITY
   182 matched at least 7 of the 16 largest peaks in the unknown

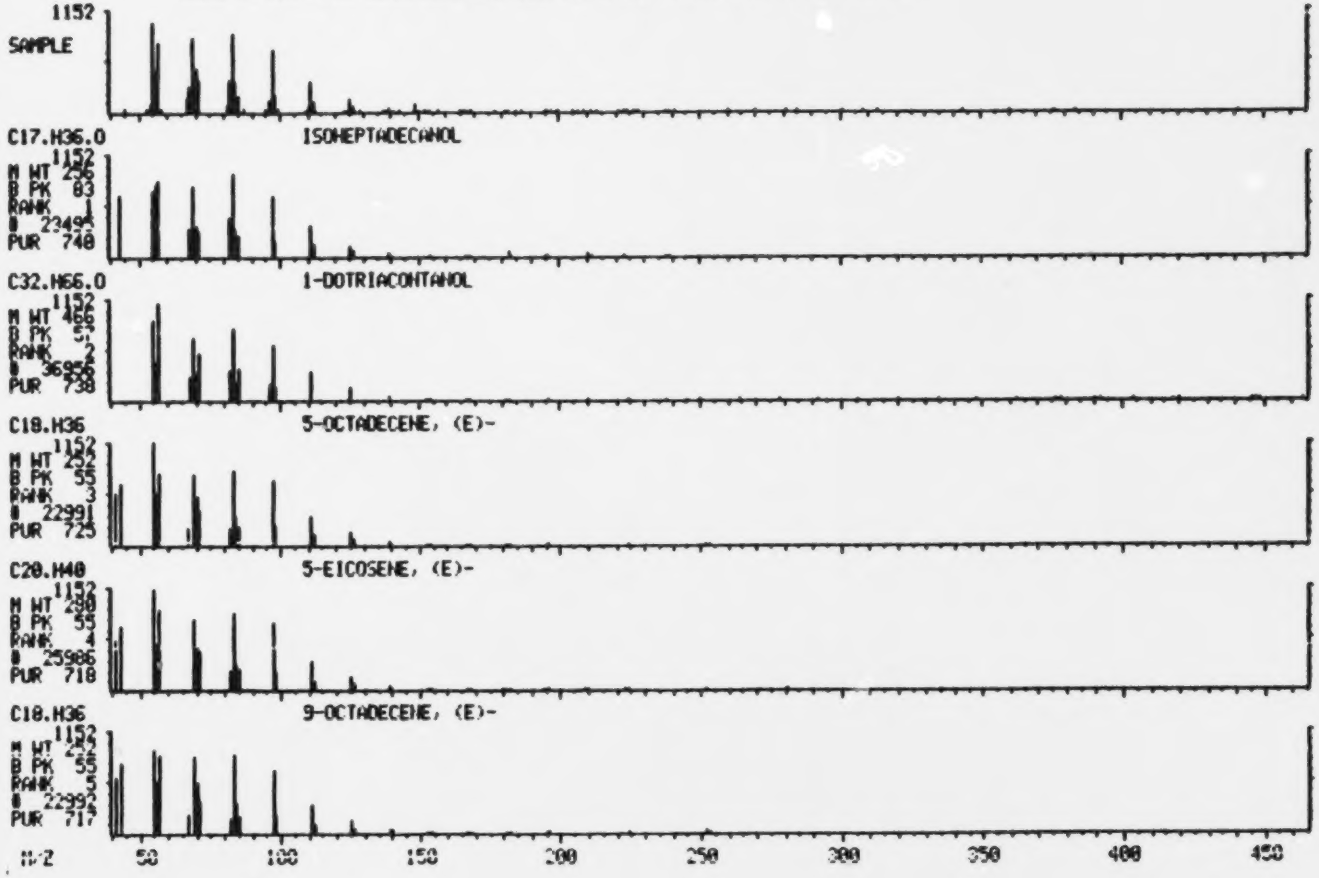
Rank In.        Name
1 23495 ISOHEPTADECANOL
2 36956 1-DOTRIACONTANOL
3 22991 5-OCTADECENE, (E)-
4 25986 5-EICOSENE, (E)-
5 22992 9-OCTADECENE, (E)-
6 15402 CYCLOTETRADECANE
7 25985 9-EICOSENE, (E)-
8 19558 1-HEXADECENE
9 19557 CYCLOHEXADECANE

Rank  Formula                                      M.Wt B.Pk   Purity    Fit   RFit
1     C17.H36.O                                     256   83      740    905    791
2     C32.H66.O                                     466   57      738    921    787
3     C18.H36                                       252   55      725    896    803
4     C20.H40                                       280   55      718    903    789
5     C18.H36                                       252   55      717    886    802
6     C14.H28                                       196   55      716    902    788
7     C20.H40                                       280   57      712    894    788
8     C16.H32                                       224   57      710    898    784
9     C16.H32                                       224   55      708    888    787

Rank     Ret.Time     B.P.Int.     US.Par.1     US.Par.2     C.A.S. #
1          ____         ____        0.7280        ____       57239-07-3
2          ____         ____        0.2480        ____        6624-79-9
3          ____         ____        0.5070        ____        7206-21-5
4          ____         ____        0.5200        ____       74685-30-6
5          ____         ____        0.5760        ____        7206-25-9
6          ____         ____        0.5840        ____         295-17-0
7          ____         ____        0.5720        ____       74685-29-3
8          ____         ____        0.6730        ____         629-73-2
9          ____         ____        0.6180        ____         295-65-8
```

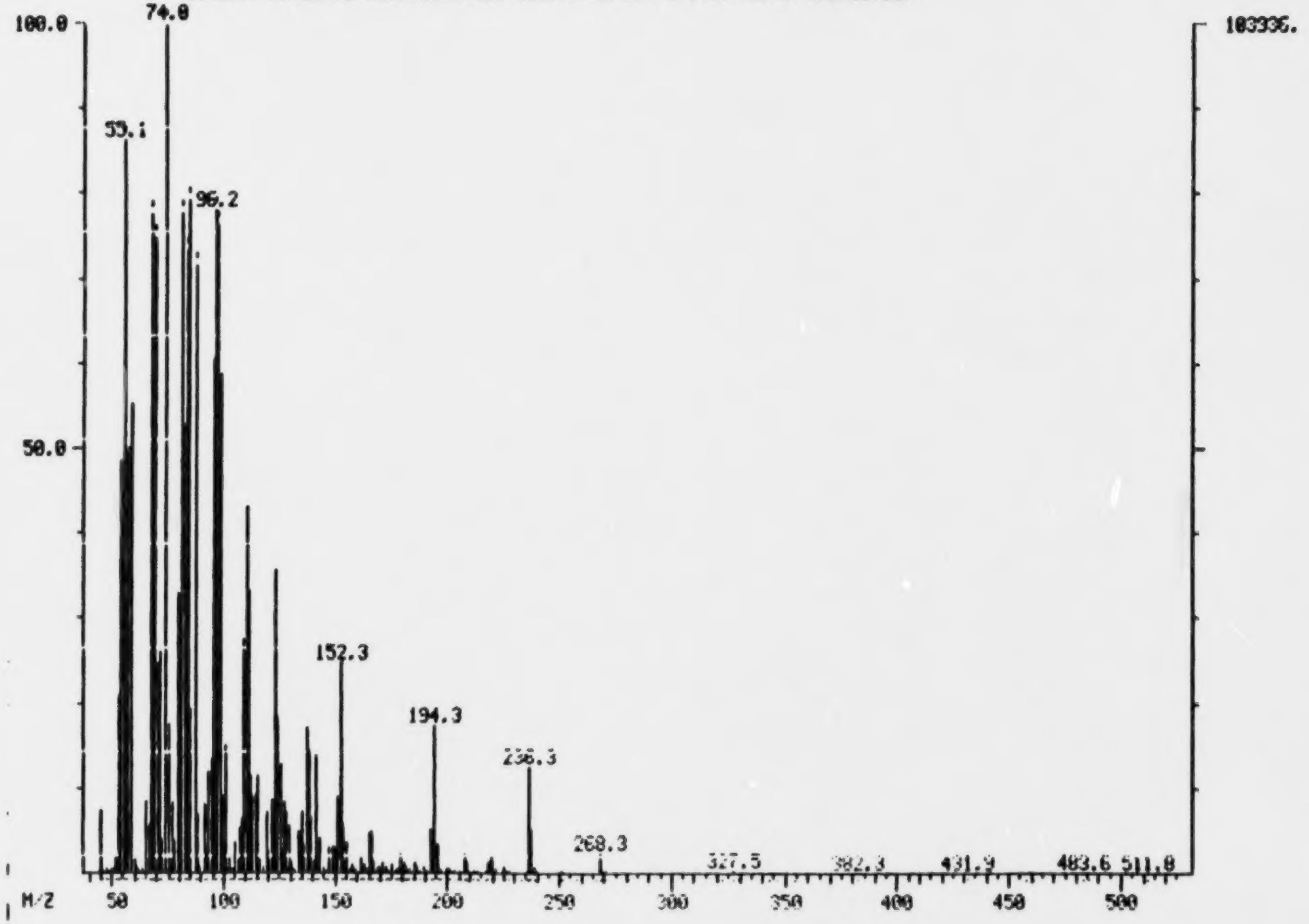
MMS SAMPLE 1
PEAK 4
LIBRARY SEARCH REPORT

```
LIBRARY SEARCH                                        DATA: 567719 #1626        BASE M/Z:  55
05/06/86 15:33:00 + 19:06                             CALI: 567610CAL32 #   4   RIC:     580607.
SAMPLE: 41702-08-01 MMS EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
```



MMS SAMPLE 1
PEAK 4
COMPARISON LIBRARY MASS SPECTRA

```
MASS SPECTRUM                                        DATA: 567719 #1646         BASE M/Z:  74
05/06/86 15:33:00 + 19:20                            CALI: 567610CAL32 #4       RIC:   2070520.
SAMPLE: 41702-08-01 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
```



MM5 SAMPLE 1
PEAK 5
MASS SPECTRUM

```
Library Search                  Data: 567719 #1646          Base m/z:    74
05/06/86 15:33:00 + 19:20       Cali: 567610CAL32 #    4    RIC:    1738750.
Sample: 41702-08-01 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

39763 spectra in LIBRARYNB searched for maximum PURITY
  412 matched at least 6 of the 16 largest peaks in the unknown

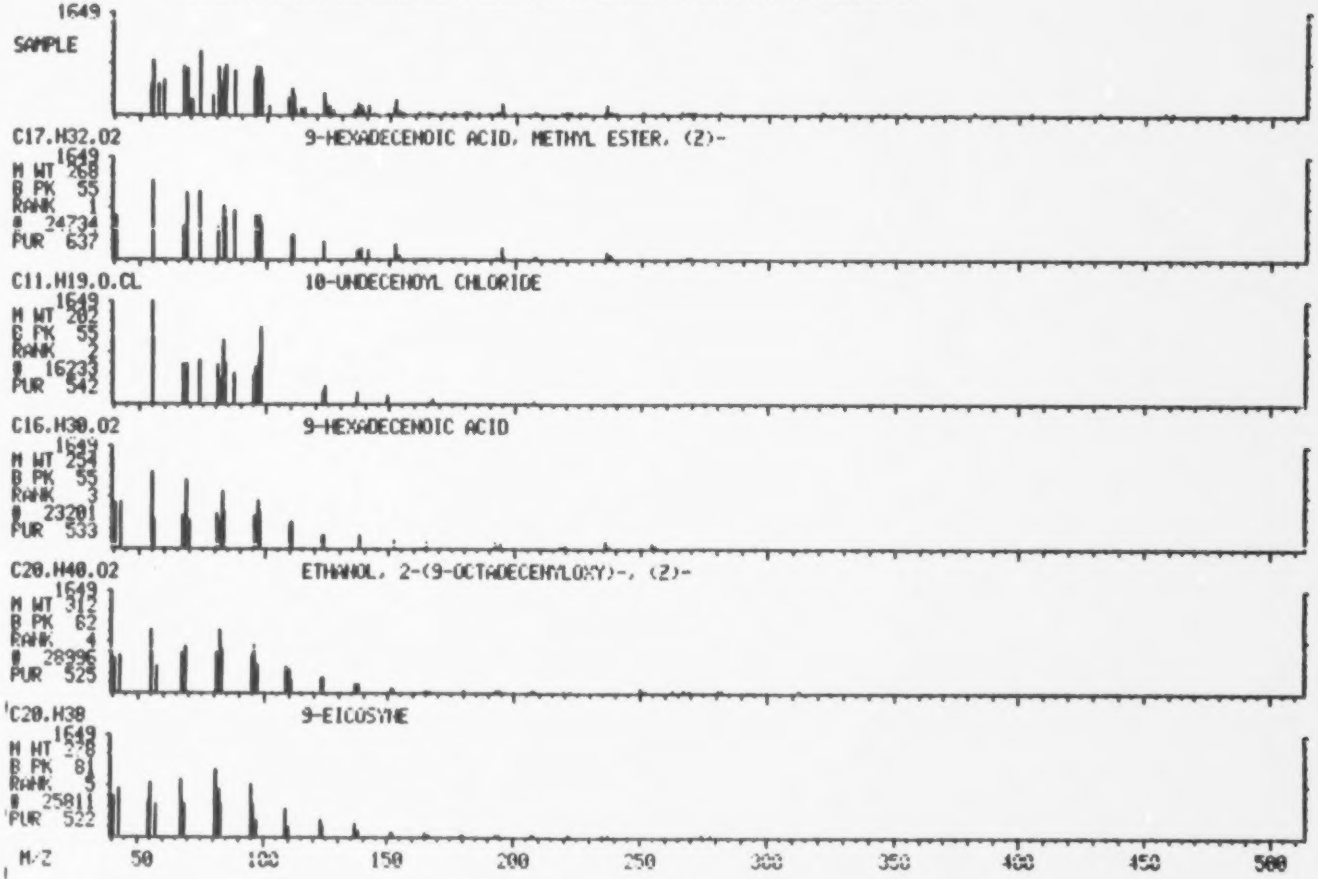
Rank In.       Name
1 24734 9-HEXADECENOIC ACID, METHYL ESTER, (Z)-
2 16233 10-UNDECENOYL CHLORIDE
3 23201 9-HEXADECENOIC ACID
4 28996 ETHANOL, 2-(9-OCTADECENYLOXY)-, (Z)-
5 25311 9-EICOSYNE
6 30024 EICOSENOIC ACID, METHYL ESTER
7 14980 1,13-TETRADECADIENE
8 23226 CYCLOHEPTADECANOL
9 28999 ETHANOL, 2-(9-OCTADECENYLOXY)-, (E)-

Rank  Formula                              M.Wt B.Pk   Purity    Fit   RFit
1     C17.H32.O2                            268   55      637    961    655
2     C11.H19.O.CL                          202   55      542    945    548
3     C16.H30.O2                            254   55      533    864    595
4     C20.H40.O2                            312   82      525    873    564
5     C20.H38                               278   81      522    867    563
6     C21.H40.O2                            324   43      514    858    579
7     C14.H26                               194   55      509    896    534
8     C17.H34.O                             254   82      505    848    545
9     C20.H40.O2                            312   82      503    869    529

Rank     Ret.Time      B.P.Int.       US.Par.1      US.Par.2       C.A.S. #
1                                     0.7130                       1120-25-8
2          ____          ____         0.3840          ____        38460-95-6
3          ____          ____         0.6330          ____         2091-29-4
4          ____          ____         0.6760          ____         5353-25-3
5          ____          ____         0.7130          ____        71899-38-2
6          ____          ____         0.6110          ____        27070-40-2
7          ____          ____         0.6100          ____        21964-49-8
8          ____          ____         0.6970          ____         4429-77-0
9          ____          ____         0.7200          ____        17367-07-6
```

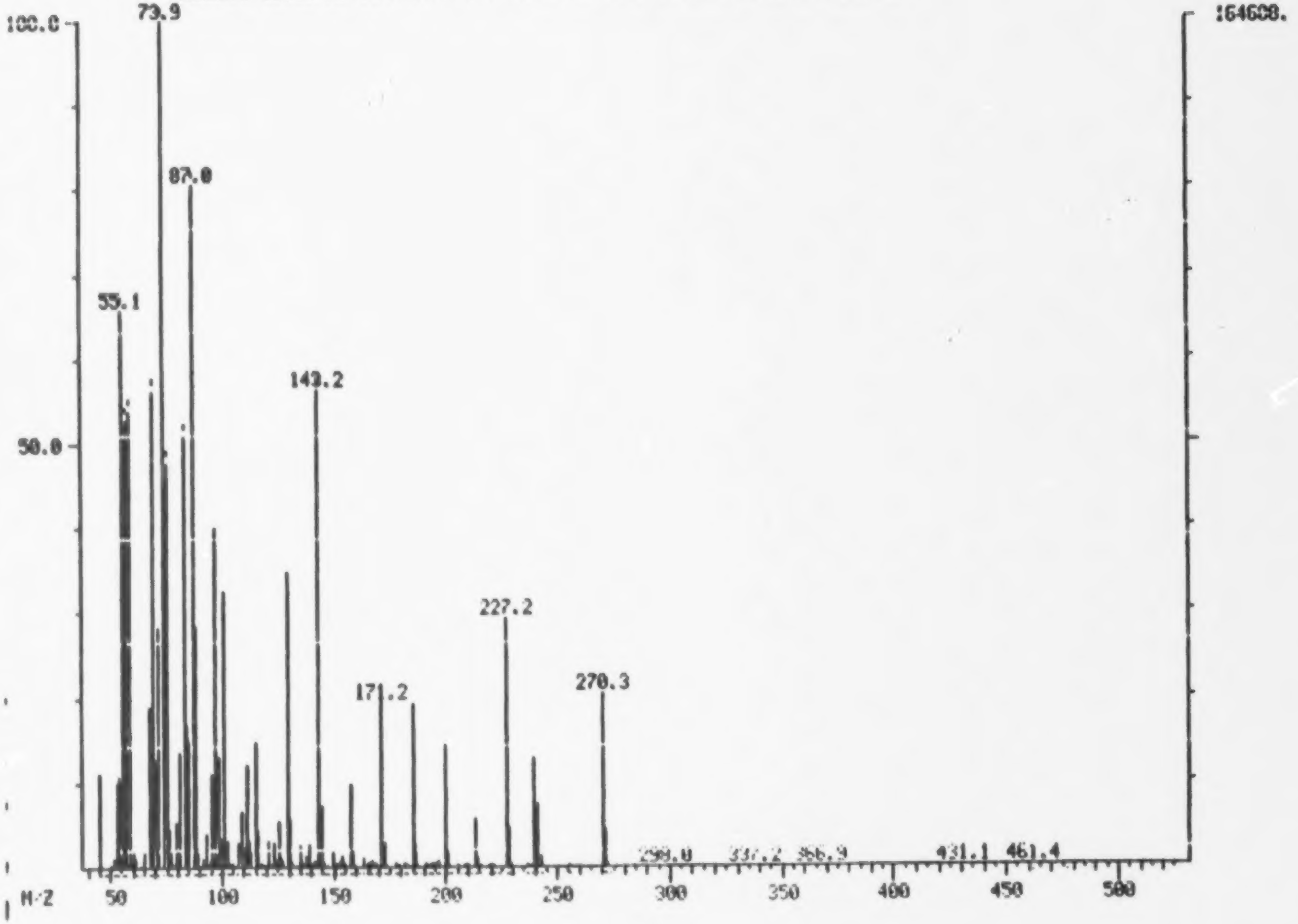
MM5 SAMPLE 1
PEAK 5
LIBRARY SEARCH REPORT

```
LIBRARY SEARCH                                    DATA: 567719 #1646         BASE M/Z:  74
05/06/86 15:33:00 + 19:20                         CALI: 567610CAL32 #   4    RIC:  1738750.
SAMPLE: 41702-08-01 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI.GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
```



MM5 SAMPLE 1
PEAK 5
COMPARISON LIBRARY MASS SPECTRA

```
MASS SPECTRUM                                                DATA: 567719 #1666        BASE M/Z:  74
05/06/86 15:33:00 + 19:34                                    CALI: 567610CAL32 #4      RIC:  1955030.
SAMPLE: 41702-08-01 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
```



MM5 SAMPLE 1
PEAK 6
MASS SPECTRUM

```
Library Search                 Data: 567719 #1666        Base m/z:    74
05/06/86 15:33:00 + 19:34      Cali: 567610CAL32 #   4   RIC:    1849340.
Sample: 41702-08-01 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
Conds : EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

39763 spectra in LIBRARYNB searched for maximum PURITY
  163 matched at least 5 of the 16 largest peaks in the unknown

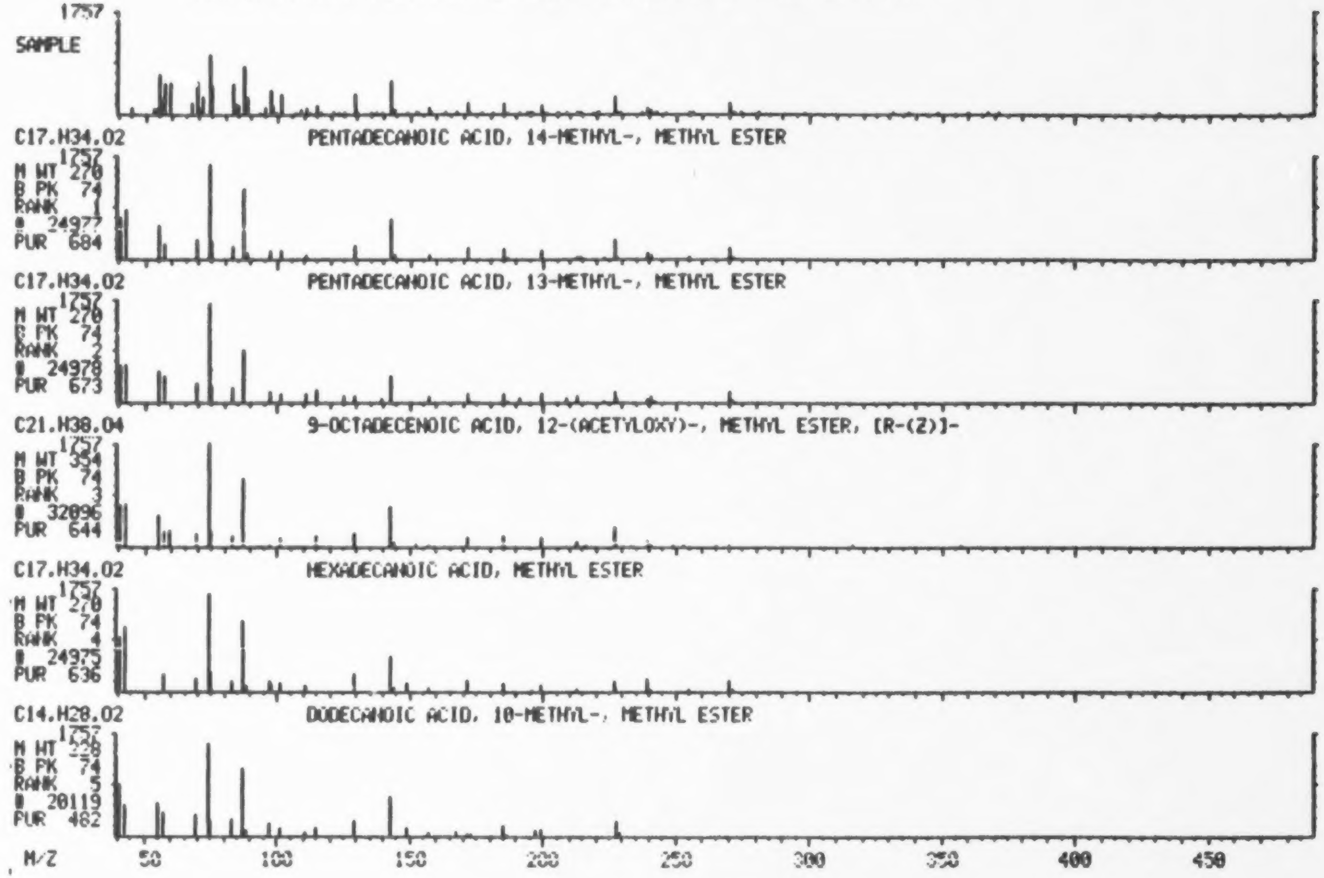
Rank In.        Name
1 24977 PENTADECANOIC ACID, 14-METHYL-, METHYL ESTER
2 24978 PENTADECANOIC ACID, 13-METHYL-, METHYL ESTER
3 32096 9-OCTADECENOIC ACID, 12-(ACETYLOXY)-, METHYL ESTER, [R-(Z)]-
4 24975 HEXADECANOIC ACID, METHYL ESTER
5 20119 DODECANOIC ACID, 10-METHYL-, METHYL ESTER
6 21308 TETRADECANOIC ACID, METHYL ESTER
7 26377 HEXADECANOIC ACID, 15-METHYL-, METHYL ESTER
8 18094 UNDECANOIC ACID, 10-METHYL-, METHYL ESTER
9 26379 TETRADECANOIC ACID, 5,9,13-TRIMETHYL-, METHYL ESTER

Rank  Formula                                  M.Wt B.Pk   Purity    Fit   RFit
1     C17.H34.O2                                270   74      684    885    741
2     C17.H34.O2                                270   74      673    878    718
3     C21.H38.O4                                354   74      644    842    709
4     C17.H34.O2                                270   74      636    848    702
5     C14.H28.O2                                228   74      482    791    540
6     C15.H30.O2                                242   74      476    764    560
7     C18.H36.O2                                284   74      467    665    613
8     C13.H26.O2                                214   74      460    777    514
9     C18.H36.O2                                284   74      456    778    513

Rank      Ret.Time      B.P.Int.      US.Par.1      US.Par.2       C.A.S. #
1          ____          ____          0.7090         ____          5129-60-2
2          ____          ____          0.6430         ____          5487-50-3
3          ____          ____          0.5950         ____           140-03-4
4          ____          ____          0.7140         ____           112-39-0
5          ____          ____          0.6300         ____          5129-65-7
6          ____          ____          0.6440         ____           124-10-7
7          ____          ____          0.6650         ____          6929-04-0
8          ____          ____          0.7210         ____          5129-56-6
9          ____          ____          0.5970         ____         56196-55-5
```

MM5 SAMPLE 1
PEAK 6
LIBRARY SEARCH REPORT

```
LIBRARY SEARCH                                          DATA: 567719 #1666        BASE M/Z:  74
05/06/86 15:33:00 + 19:34                               CALI: 567610CAL32 #   4    RIC:  1849340.
SAMPLE: 41702-08-01 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
```

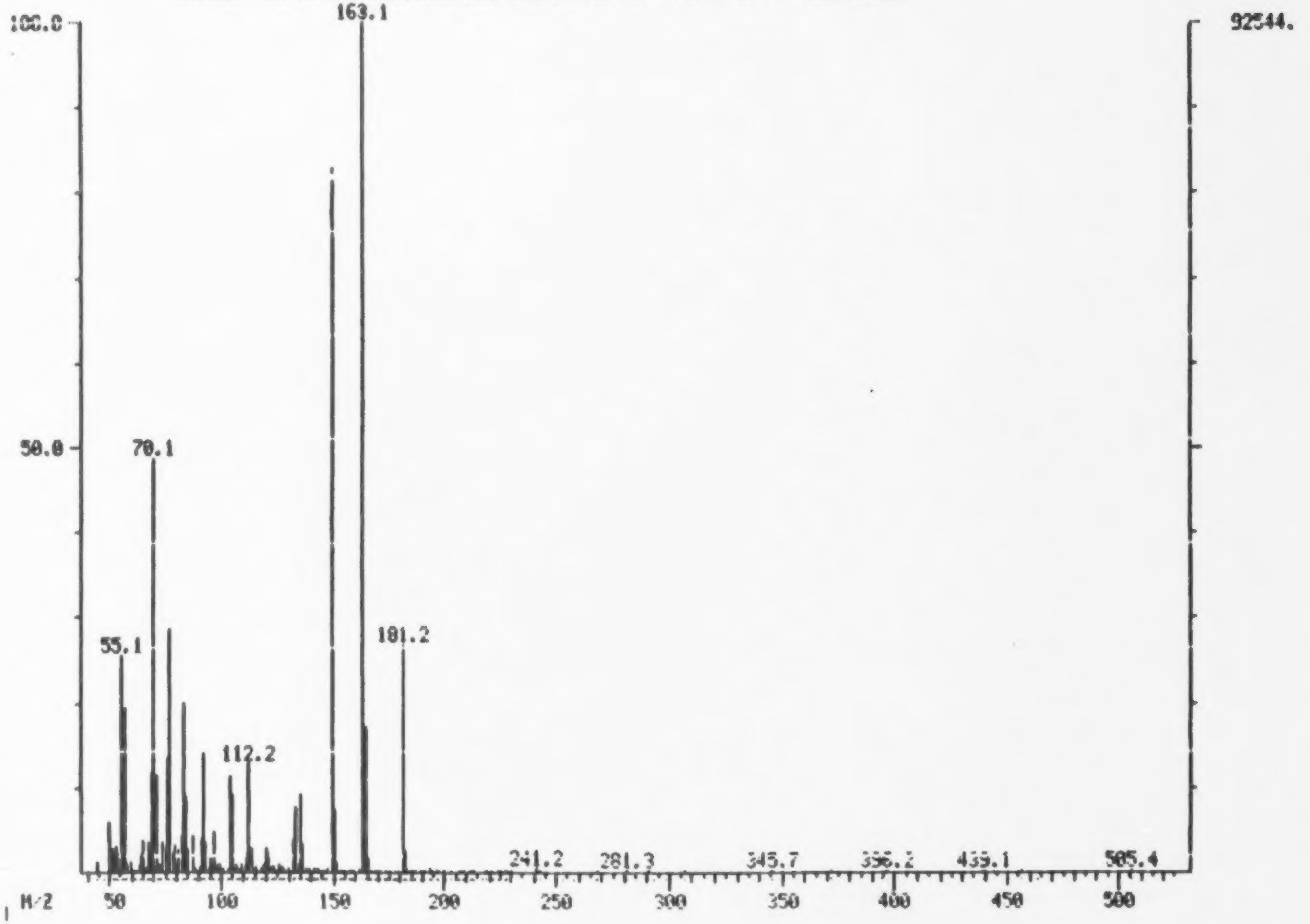


MM5 SAMPLE 1
PEAK 6
COMPARISON LIBRARY MASS SPECTRA

MASS SPECTRUM
05/06/86 15:33:00 + 20:36
SAMPLE: 41702-08-01 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

DATA: 567719 #1754
CALI: 567610CAL32 #4

BASE M/Z: 163
RIC: 576512.



MM5 SAMPLE 1
PEAK 7
MASS SPECTRUM

```
Library Search                 Data: 567719 #1754          Base m/z:  163
05/06/86 15:33:00 + 20:36      Cali: 567610CAL32 #   4     RIC:     526335.
Sample: 41702-08-01 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

39763 spectra in LIBRARYNB searched for maximum PURITY
   544 matched at least 4 of the 16 largest peaks in the unknown

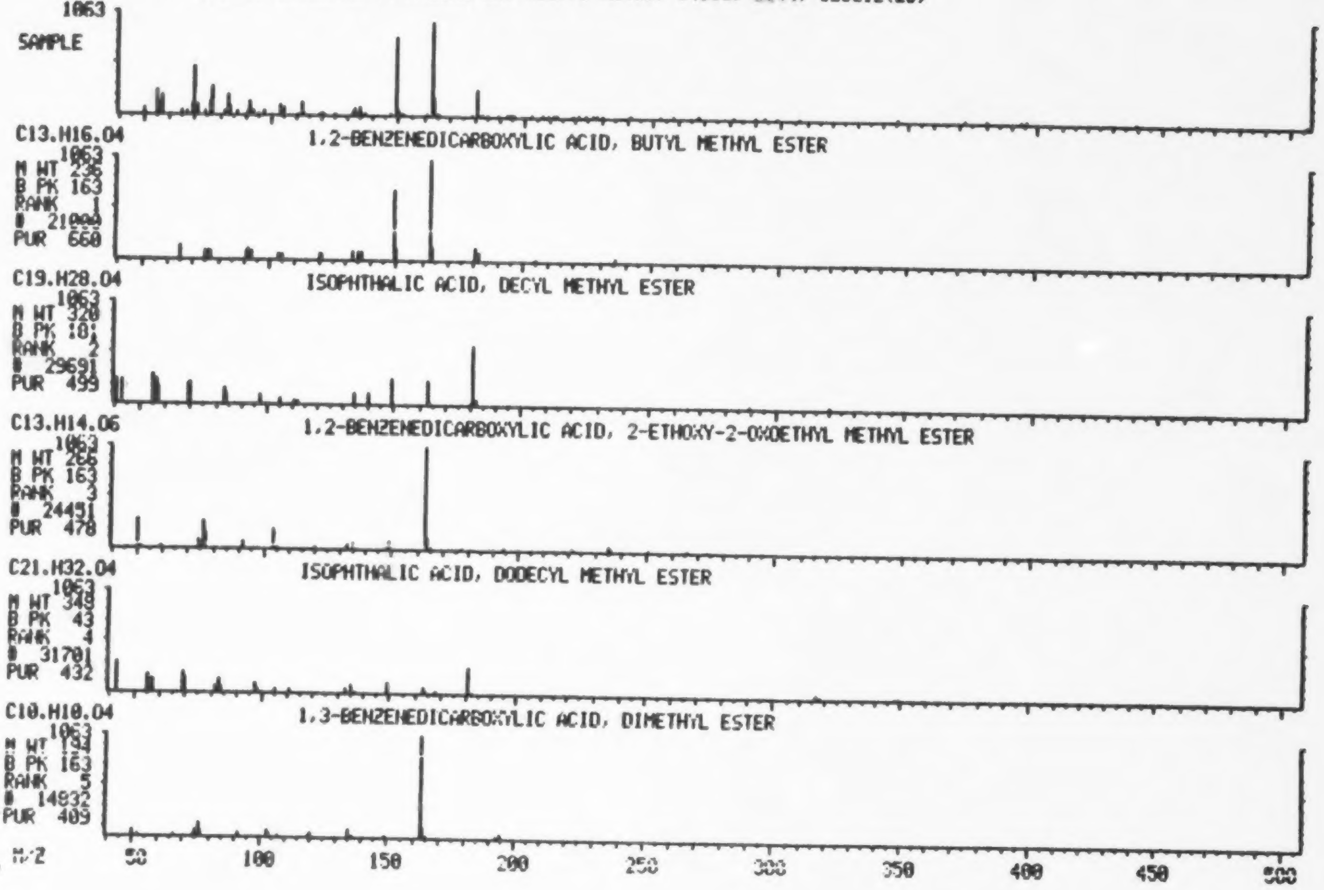
Rank In.        Name
1 21000 1,2-BENZENEDICARBOXYLIC ACID, BUTYL METHYL ESTER
2 29691 ISOPHTHALIC ACID, DECYL METHYL ESTER
3 24451 1,2-BENZENEDICARBOXYLIC ACID, 2-ETHOXY-2-OXOETHYL METHYL ESTER
4 31701 ISOPHTHALIC ACID, DODECYL METHYL ESTER
5 14832 1,3-BENZENEDICARBOXYLIC ACID, DIMETHYL ESTER
6 14824 1,2-BENZENEDICARBOXYLIC ACID, DIMETHYL ESTER
7 16833 TEREPHTHALIC ACID, METHYL VINYL ESTER
8  9899 1,3-BENZENEDIAMINE, N,N'-DIETHYL-
9  9805 1H-ISOINDOLE-1,3(2H)-DIONE, 2-HYDROXY-

Rank   Formula                                M.Wt B.Pk   Purity    Fit   RFit
1      C13.H16.O4                              236  163      660    910    691
2      C19.H28.O4                              320  181      499    674    586
3      C13.H14.O6                              266  163      478    763    524
4      C21.H32.O4                              348   43      432    567    548
5      C10.H10.O4                              194  163      409    683    436
6      C10.H10.O4                              194  163      402    895    421
7      C11.H10.O4                              206  163      395    643    444
8      C10.H16.N2                              164  149      383    662    400
9      C8.H5.O3.N                              163  163      355    878    369

Rank      Ret Time      B.P.Int.       US.Par.1      US.Par.2        C.A.S. #
1           ____          ____          0.4910         ____          34006-76-3
2           ____          ____          0.4110         ____          33975-27-8
3           ____          ____          0.6910         ____             85-71-2
4           ____          ____          0.4440         ____          33975-26-7
5           ____          ____          0.5650         ____           1459-93-4
6           ____          ____          0.5350         ____            131-11-3
7           ____          ____          0.4890         ____           4091-02-5
8           ____          ____          0.6120         ____           5857-99-8
9           ____          ____          0.6110         ____            524-38-9
```

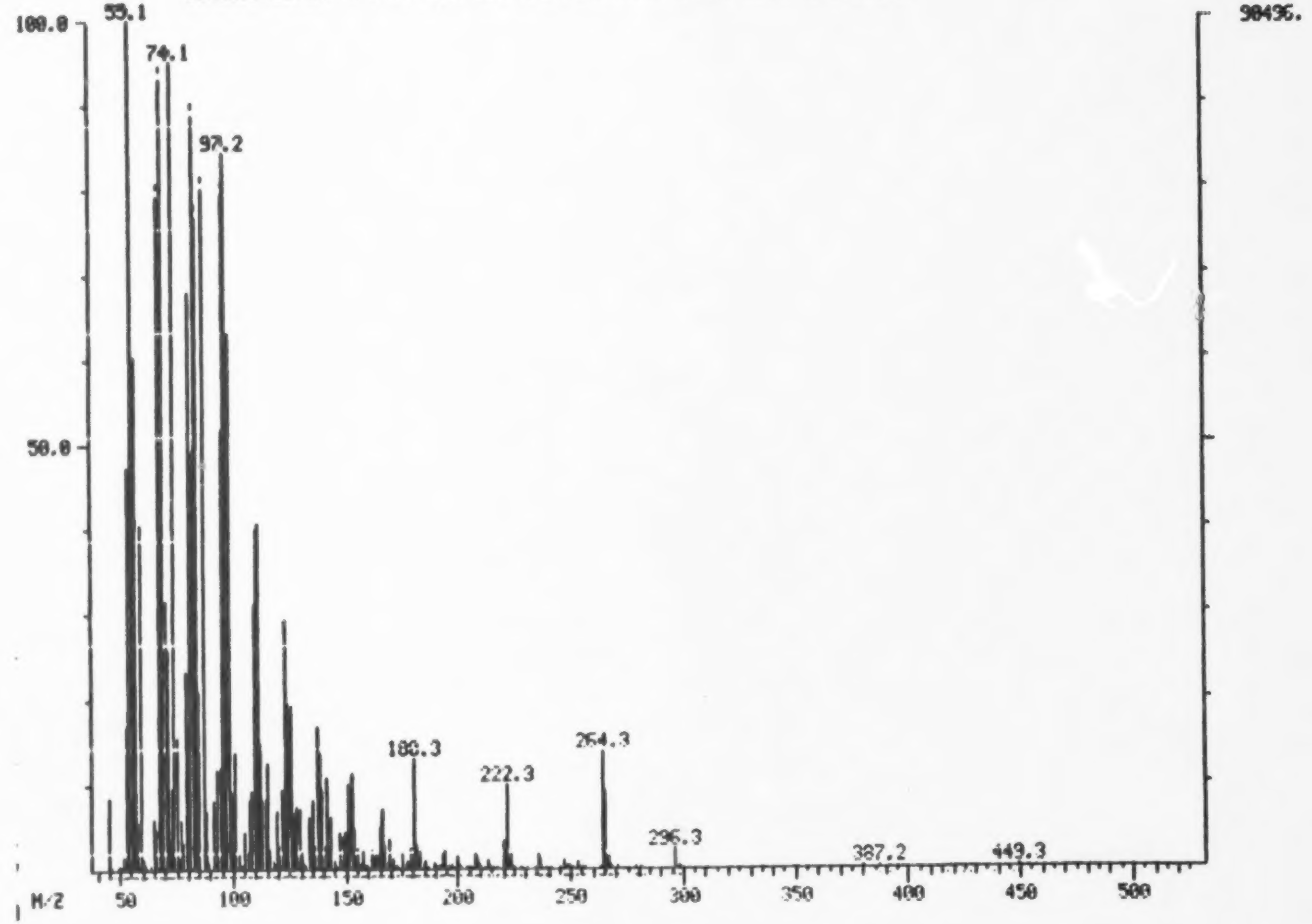
MMS SAMPLE 1
PEAK 7
LIBRARY SEARCH REPORT

```
LIBRARY SEARCH                                              DATA: 567719 #1754          BASE M/Z: 163
05/06/86 15:33:00 + 20:36                                   CALI: 567610CAL32 #   4     RIC:   526335.
SAMPLE: 41702-08-01 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI.GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
```



MM5 SAMPLE 1
PEAK 7
COMPARISON LIBRARY MASS SPECTRA

MASS SPECTRUM DATA: 567719 #1790 BASE M/Z: 55
05/06/86 15:33:00 + 21:07 CALI: 567610CAL32 #4 RIC: 1867770.
SAMPLE: 41702-08-01 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)



MM5 SAMPLE 1
PEAK 8
MASS SPECTRUM

```
Library Search                 Data: 567719 #1798        Base m/z:   55
05/06/86 15:33:00 + 21:07      Cali: 567610CAL32 #   4   RIC:    1562620
Sample: 41702-08-01 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

39763 spectra in LIBRARYNB searched for maximum PURITY
  394 matched at least 6 of the 16 largest peaks in the unknown

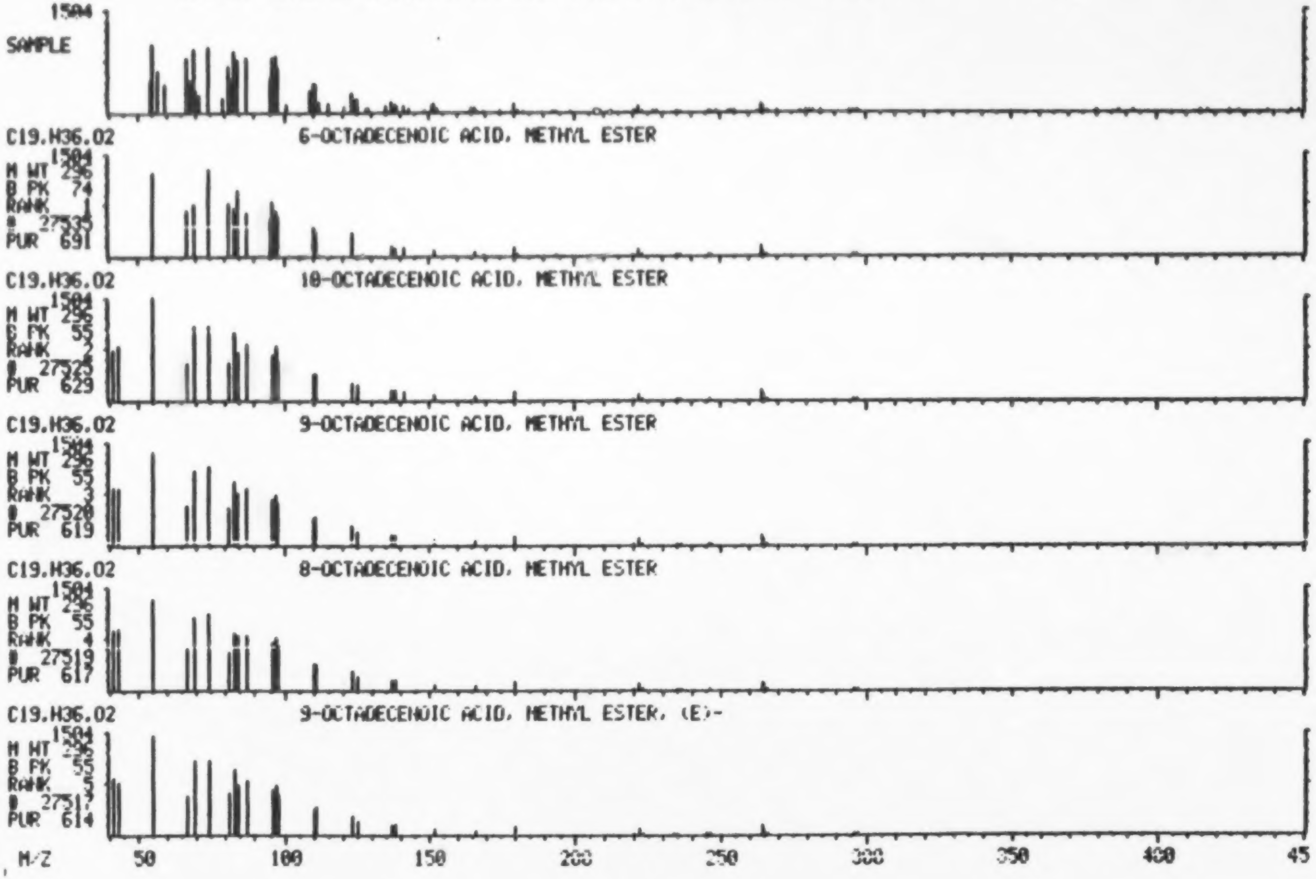
Rank In.       Name
1 27535 6-OCTADECENOIC ACID, METHYL ESTER
2 27525 10-OCTADECENOIC ACID, METHYL ESTER
3 27520 9-OCTADECENOIC ACID, METHYL ESTER
4 27519 8-OCTADECENOIC ACID, METHYL ESTER
5 27517 9-OCTADECENOIC ACID, METHYL ESTER, (E)-
6 27518 11-OCTADECENOIC ACID, METHYL ESTER, (Z)-
7 27546 7-OCTADECENOIC ACID, METHYL ESTER
8 27538 12-OCTADECENOIC ACID, METHYL ESTER
9 27539 13-OCTADECENOIC ACID, METHYL ESTER

Rank  Formula                              M.Wt B.Pk   Purity    Fit   RFit
1     C19.H36.O2                            296   74      691    993    691
2     C19.H36.O2                            296   55      629    938    666
3     C19.H36.O2                            296   55      619    934    659
4     C19.H36.O2                            296   55      617    933    659
5     C19.H36.O2                            296   55      614    932    656
6     C19.H36.O2                            296   55      602    946    633
7     C19.H36.O2                            296   55      600    928    644
8     C19.H36.O2                            296   55      597    949    625
9     C19.H36.O2                            296   55      591    952    615

Rank    Ret.Time     B.P.Int.     US.Par.1     US.Par.2      C.A.S. #
1         ___          ___         0.7190        ___        52355-31-4
2         ___          ___         0.6890        ___        13431-95-3
3         ___          ___         0.7260        ___         2462-84-2
4         ___          ___         0.7200        ___         2345-29-1
5         ___          ___         0.7260        ___         1937-62-8
6         ___          ___         0.7200        ___         1937-63-9
7         ___          ___         0.7270        ___        57396-98-2
8         ___          ___         0.7180        ___        56554-46-2
9         ___          ___         0.6580        ___        56554-47-3
```

MM5 SAMPLE 1
PEAK 8
LIBRARY SEARCH REPORT

```
LIBRARY SEARCH                                           DATA: 567719 #1798        BASE M/Z:  55
05/06/86 15:33:00 + 21:07                                CALI: 567610CAL32 #   4   RIC:  1562620.
SAMPLE: 41702-08-01 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI.GC/MS 30MX.25MM DB5 #30779 HE(30) I(300) 60(4)-320@12(20)
```

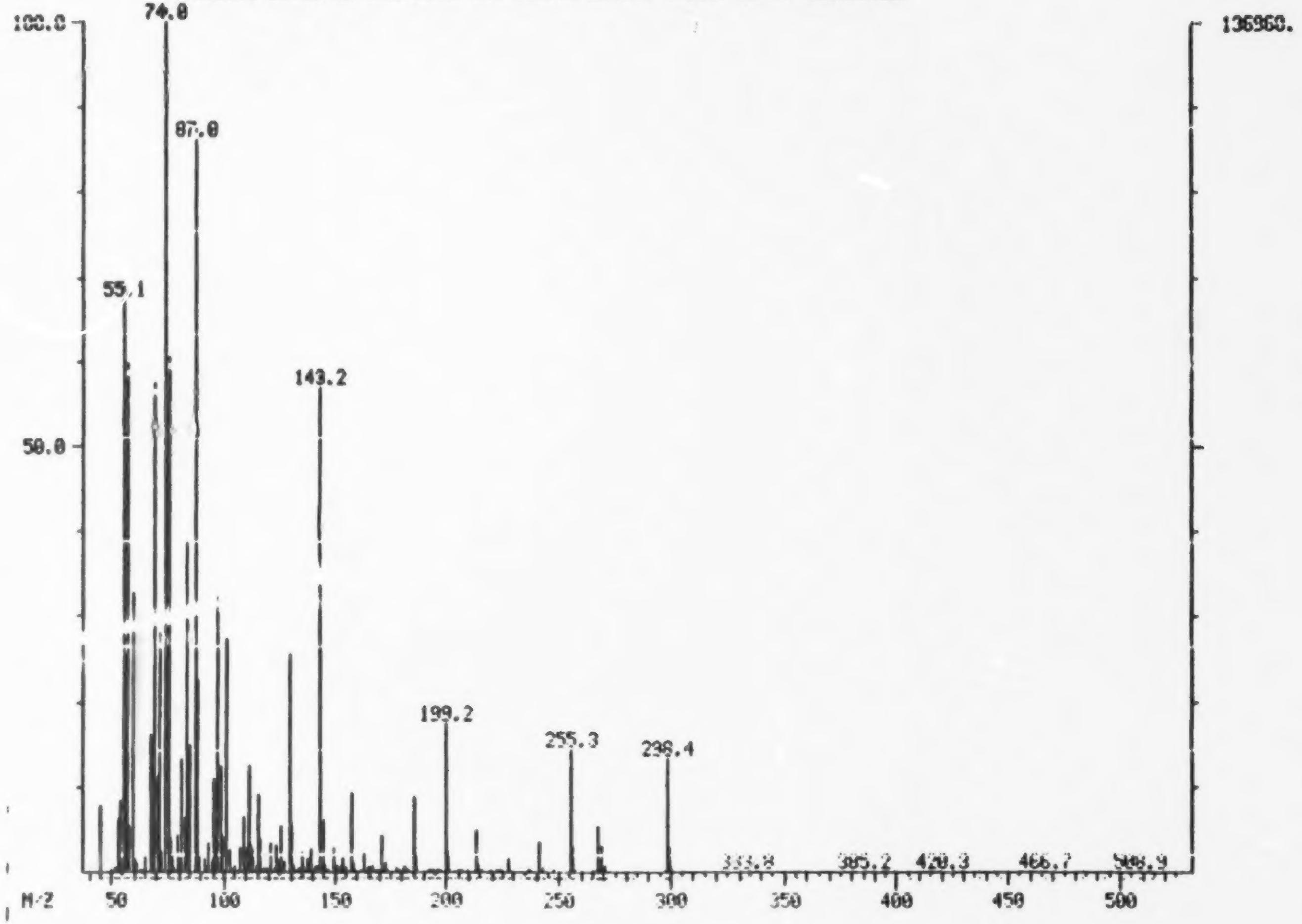


MM5 SAMPLE 1
PEAK 8
COMPARISON LIBRARY MASS SPECTRA

MASS SPECTRUM
05/06/86 15:33:00 + 21:20
SAMPLE: 41702-08-01 MMS EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

DATA: 567719 #1816
CALI: 567610CAL32 #4

BASE M/Z: 74
RIC: 1482750.



MMS SAMPLE 1
PEAK 9
MASS SPECTRUM

```
Library Search              Data: 567719 #1816          Base m/z:    74
05/06/86 15.33:00 + 21:20   Cali: 567610CAL32 #   4   RIC:    1347580.
Sample: 41702-08-01 MMS EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

39763 spectra in LIBRARYNB searched for maximum PURITY
  127 matched at least 6 of the 16 largest peaks in the unknown

Rank In.      Name
1 27752 HEPTADECANOIC ACID, 15-METHYL-, METHYL ESTER
2 27747 HEPTADECANOIC ACID, 16-METHYL-, METHYL ESTER
3 27745 HEPTADECANOIC ACID, 14-METHYL-, METHYL ESTER
4 27759 HEPTADECANOIC ACID, 14-METHYL-, METHYL ESTER, (.+-.)-
5 27746 HEPTADECANOIC ACID, 10-METHYL-, METHYL ESTER
6 27754 HEPTADECANOIC ACID, 9-METHYL-, METHYL ESTER
7 36952 TRIACONTANOIC ACID, METHYL ESTER
8 27741 OCTADECANOIC ACID, METHYL ESTER
9 21808 TETRADECANOIC ACID, METHYL ESTER

Rank  Formula                                  M.Wt B.Pk   Purity   Fit   RFit
1     C19.H38.O2                                298   74      698   927    726
2     C19.H38.O2                                298   74      697   908    737
3     C19.H38.O2                                298   74      641   846    676
4     C19.H38.O2                                298   74      640   846    675
5     C19.H38.O2                                298   74      630   832    676
6     C19.H38.O2                                298   74      596   797    694
7     C31.H62.O2                                466   74      520   770    644
8     C19.H38.O2                                298   74      519   753    607
9     C15.H30.O2                                242   74      511   786    605

Rank    Ret.Time     B.P.Int.    US.Par.1    US.Par.2      C.A.S. #
1        ____         ____        0.7130       ____      54833-55-5
2        ____         ____        0.6960       ____        5129-61-3
3        ____         ____        0.7110       ____        2490-23-5
4        ____         ____        0.7070       ____      57274-45-0
5        ____         ____        0.6670       ____        2490-25-7
6        ____         ____        0.7140       ____      54934-57-5
7        ____         ____        0.3330       ____         629-83-4
8        ____         ____        0.7170       ____         112-61-8
9        ____         ____        0.6440       ____         124-10-7
```

MMS SAMPLE 1
PEAK 9
LIBRARY SEARCH REPORT

```
LIBRARY SEARCH                                    DATA: 567719 #1816       BASE M/Z:  74
05/06/86 15:33:00 + 21:20                         CALI: 567610CAL32 #   4  RIC:  1347580.
SAMPLE: 41702-08-01 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
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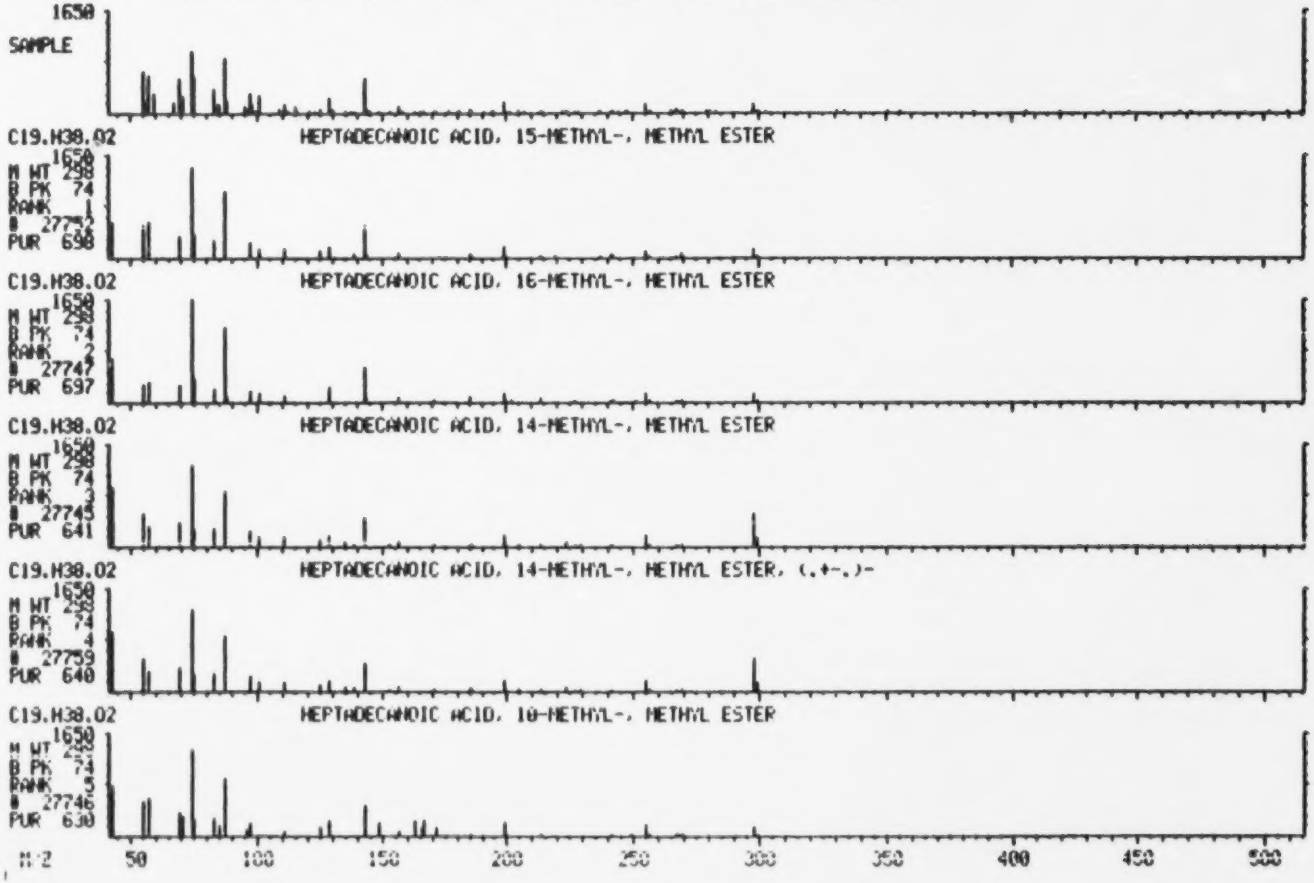
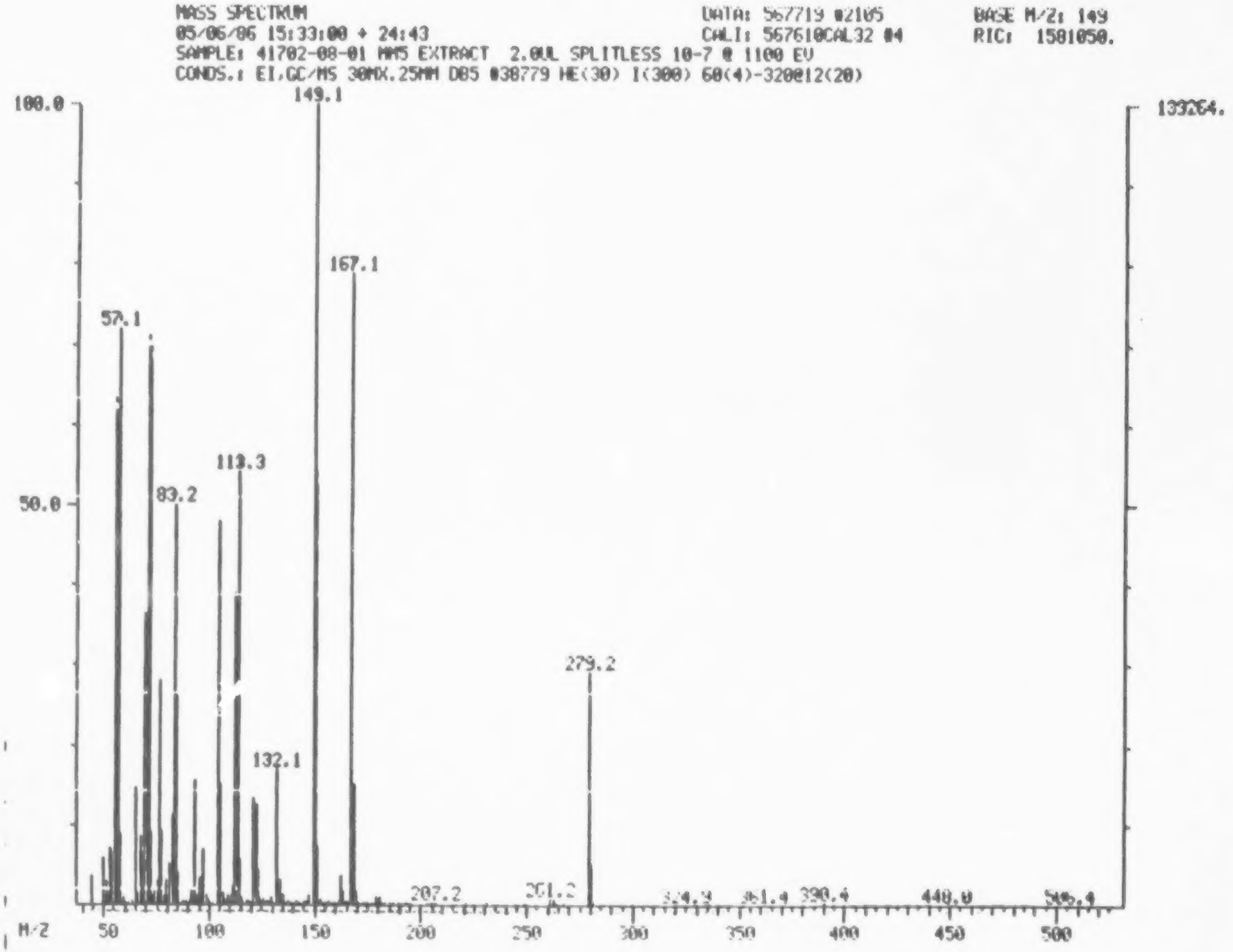


MM5 SAMPLE 1
PEAK 9
COMPARISON LIBRARY MASS SPECTRA



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MASS SPECTRUM                                            DATA: 567719 #2105          BASE M/Z: 149
05/06/86 15:33:00 + 24:43                                CALI: 567610CAL32 #4        RIC:  1581050.
SAMPLE: 41702-08-01 MMS EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
```

MMS SAMPLE 1
PEAK 10
MASS SPECTRUM

```
Library Search                     Data: 567719 #2105          Base m/z:  149
05/06/86 15:33:00 + 24:43          Cali: 567610CAL32 #   4     RIC:    1466360.
Sample: 41702-08-01 MMS EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

39763 spectra in LIBRARYNB searched for maximum PURITY
  199 matched at least 6 of the 16 largest peaks in the unknown

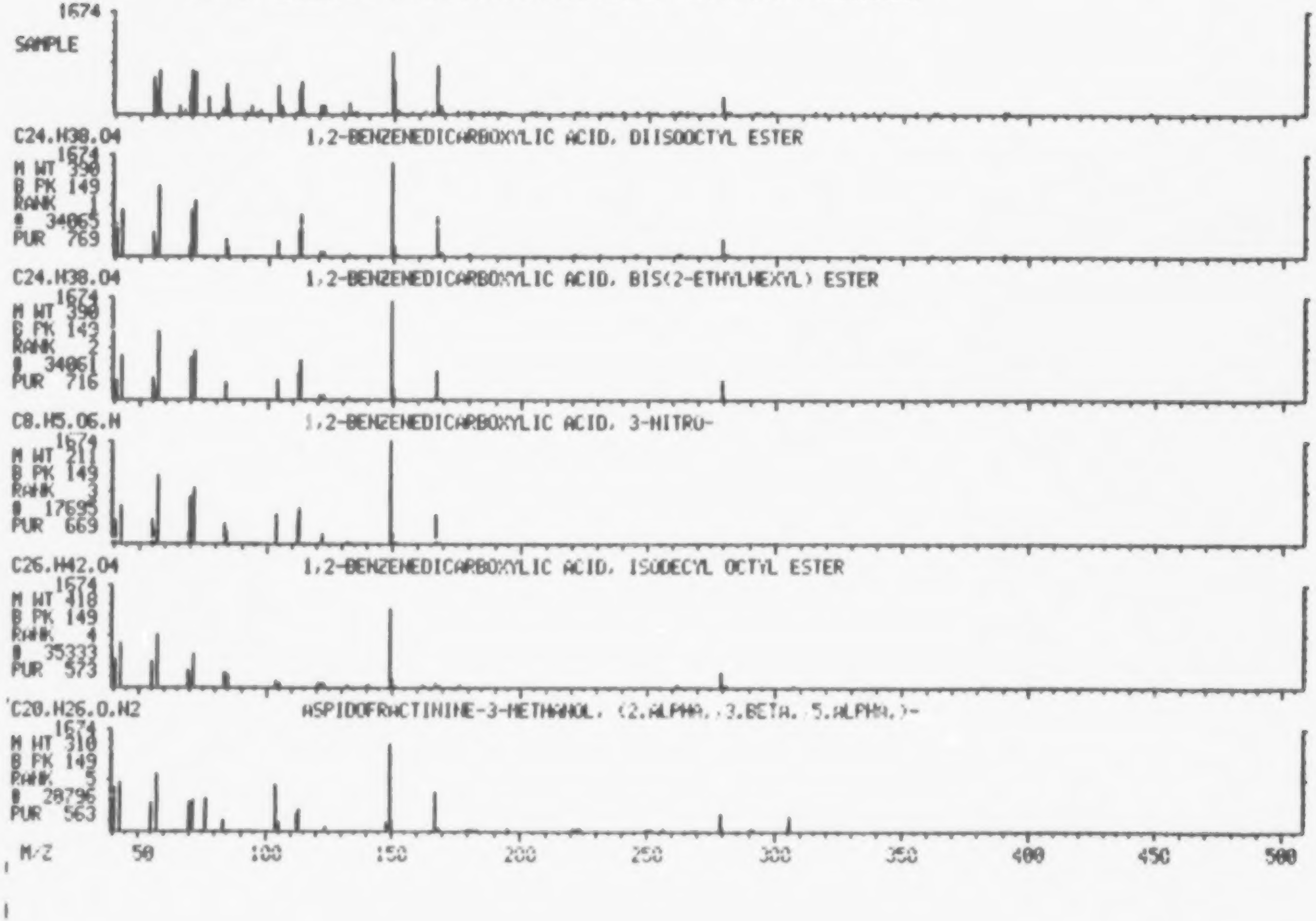
Rank In.       Name
1 34065 1,2-BENZENEDICARBOXYLIC ACID, DIISOOCTYL ESTER
2 34061 1,2-BENZENEDICARBOXYLIC ACID, BIS(2-ETHYLHEXYL) ESTER
3 17695 1,2-BENZENEDICARBOXYLIC ACID, 3-NITRO-
4 35333 1,2-BENZENEDICARBOXYLIC ACID, ISODECYL OCTYL ESTER
5 28796 ASPIDOFRACTININE-3-METHANOL, (2.ALPHA.,3.BETA.,5.ALPHA.)-
6 34062 1,2-BENZENEDICARBOXYLIC ACID, DIOCTYL ESTER
7 21976 PROPANEDIOIC ACID, HEXYL-, DIETHYL ESTER
8 21834 OCTANE, 1,1'-OXYBIS-
9 10723 1-DECENE, 3,4-DIMETHYL-

Rank  Formula                                M.Wt B.Pk   Purity    Fit   RFit
1     C24.H38.O4                              390  149      769    887    816
2     C24.H38.O4                              390  149      716    882    755
3     C8.H5.O6.N                              211  149      669    884    704
4     C26.H42.O4                              418  149      573    741    627
5     C20.H26.O.N2                            310  149      563    731    712
6     C24.H38.O4                              390  149      561    738    680
7     C13.H24.O4                              244   57      276    775    335
8     C16.H34.O                               242   57      268    759    319
9     C12.H24                                 168   57      259    689    314

Rank     Ret.Time      B.P.Int.     US.Par.1     US.Par.2      C.A.S. #
1          ___            ___        0.3270         ___        27554-26-3
2          ___            ___        0.2010         ___          117-81-7
3          ___            ___        0.8720         ___          603-11-2
4          ___            ___        0.2700         ___         1330-96-7
5          ___            ___         ___           ___         2656-44-2
6          ___            ___        0.6220         ___          117-84-0
7          ___            ___        0.4660         ___         5398-10-7
8          ___            ___        0.6370         ___          629-82-3
9          ___            ___        0.5170         ___        50871-03-9
```

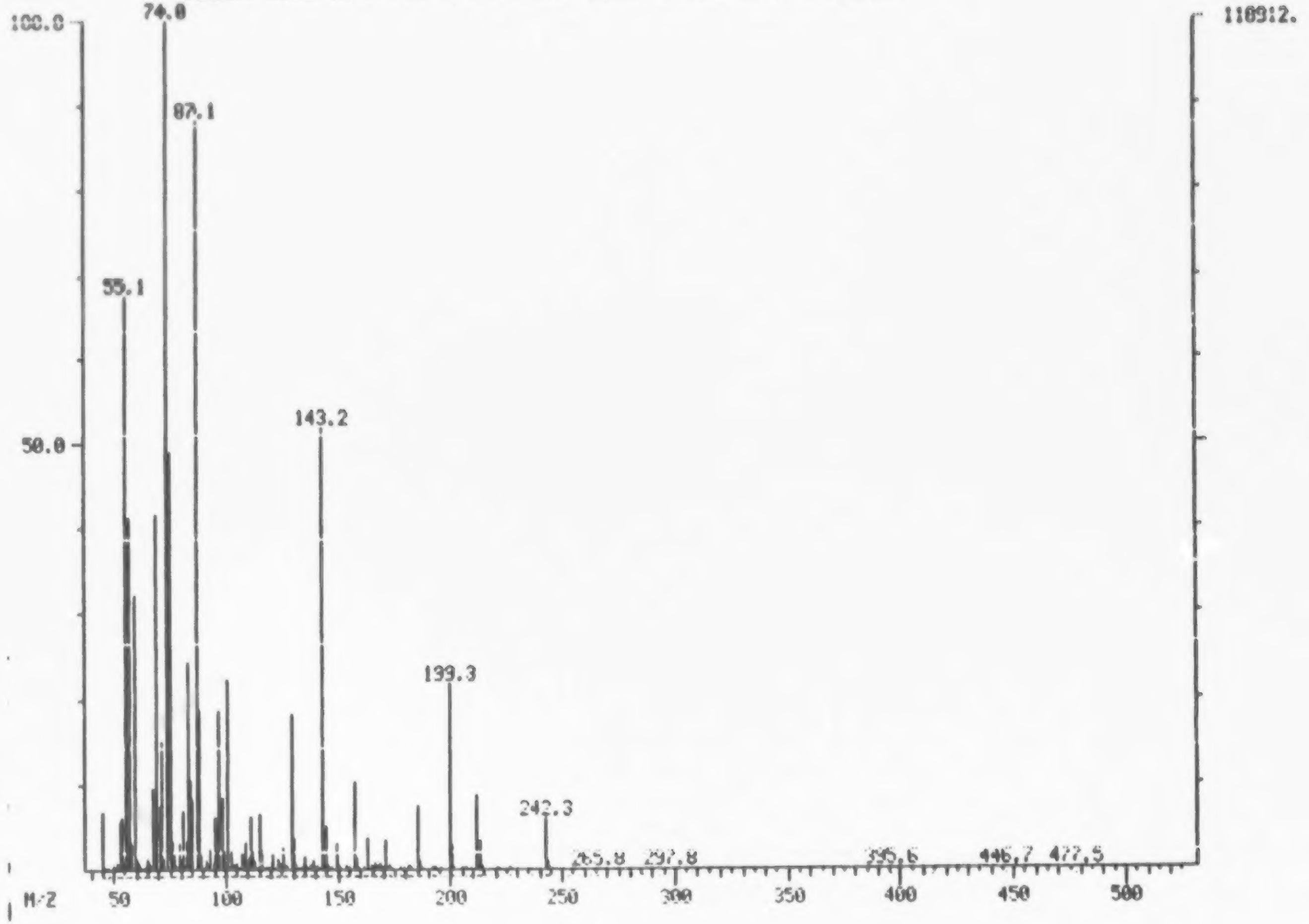
MMS SAMPLE 1
PEAK 10
LIBRARY SEARCH REPORT

```
LIBRARY SEARCH                                        DATA: 567719 #2105           BASE M/Z: 149
05/06/86 15:33:00 + 24:43                             CALI: 567610CAL32 #   4      RIC:  1466360.
SAMPLE: 41702-08-01 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
```



MM5 SAMPLE 1
PEAK 10
COMPARISON LIBRARY MASS SPECTRA

```
MASS SPECTRUM                                                DATA: 567720 01496        BASE M/Z:  74
05/06/86 16:28:00 + 17:34                                    CALI: 567610CAL32 #4      RIC:  1009660.
SAMPLE: 41702-08-02 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI.GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
```



MM5 SAMPLE 2
PEAK 1
MASS SPECTRUM

```
Library Search                 Data: 567720 01496         Base m/z:    74
05/06/86 16:28:00 + 17:34      Cali: 567610CAL32 #   4    RIC:     944127.
Sample: 41702-08-02 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

39763 spectra in LIBRARYNB searched for maximum PURITY
  457 matched at least 5 of the 16 largest peaks in the unknown

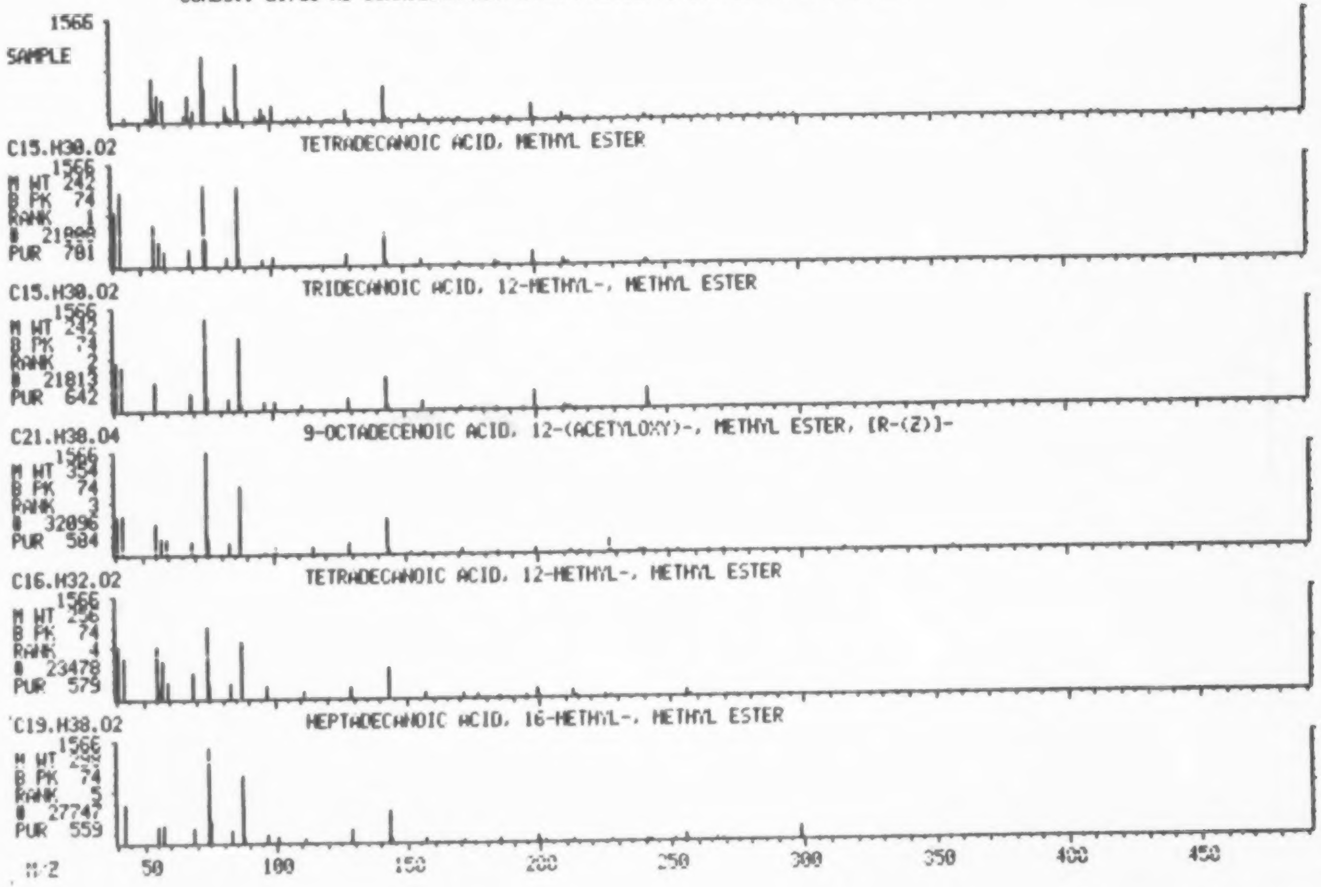
Rank In.        Name
1 21808 TETRADECANOIC ACID, METHYL ESTER
2 21813 TRIDECANOIC ACID, 12-METHYL-, METHYL ESTER
3 32096 9-OCTADECENOIC ACID, 12-(ACETYLOXY)-, METHYL ESTER, [R-(Z)]-
4 23478 TETRADECANOIC ACID, 12-METHYL-, METHYL ESTER
5 27747 HEPTADECANOIC ACID, 16-METHYL-, METHYL ESTER
6 36952 TRIACONTANOIC ACID, METHYL ESTER
7 15972 UNDECANOIC ACID, METHYL ESTER
8 18094 UNDECANOIC ACID, 10-METHYL-, METHYL ESTER
9 27746 HEPTADECANOIC ACID, 10-METHYL-, METHYL ESTER

Rank  Formula                                  M.Wt B.Pk   Purity    Fit   RFit
1     C15.H30.O2                                242   74      701    864    799
2     C15.H30.O2                                242   74      642    844    708
3     C21.H38.O4                                354   74      584    785    655
4     C16.H32.O2                                256   74      579    804    695
5     C19.H38.O2                                298   74      559    734    656
6     C31.H62.O2                                466   74      551    765    696
7     C12.H24.O2                                200   74      543    793    593
8     C13.H26.O2                                214   74      534    770    603
9     C19.H38.O2                                298   74      533    739    625

Rank     Ret.Time     B.P.Int.     US.Par.1     US.Par.2      C.A.S. #
1         ____         ____         0.6440        ____         124-10-7
2         ____         ____         0.7180        ____        5129-58-8
3         ____         ____         0.5950        ____         140-03-4
4         ____         ____         0.6840        ____        5129-66-8
5         ____         ____         0.6960        ____        5129-61-3
6         ____         ____         0.3330        ____         629-83-4
7         ____         ____         0.6270        ____        1731-86-8
8         ____         ____         0.7210        ____        5129-56-6
9         ____         ____         0.6670        ____        2490-25-7
```

MM5 SAMPLE 2
PEAK 1
LIBRARY SEARCH REPORT

```
LIBRARY SEARCH                                          DATA: 567720 #1436         BASE M/Z:  74
05/06/86 16:28:00 + 17:34                               CALI: 567610CAL32 #    4   RIC:    944127.
SAMPLE: 41702-08-02 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
```

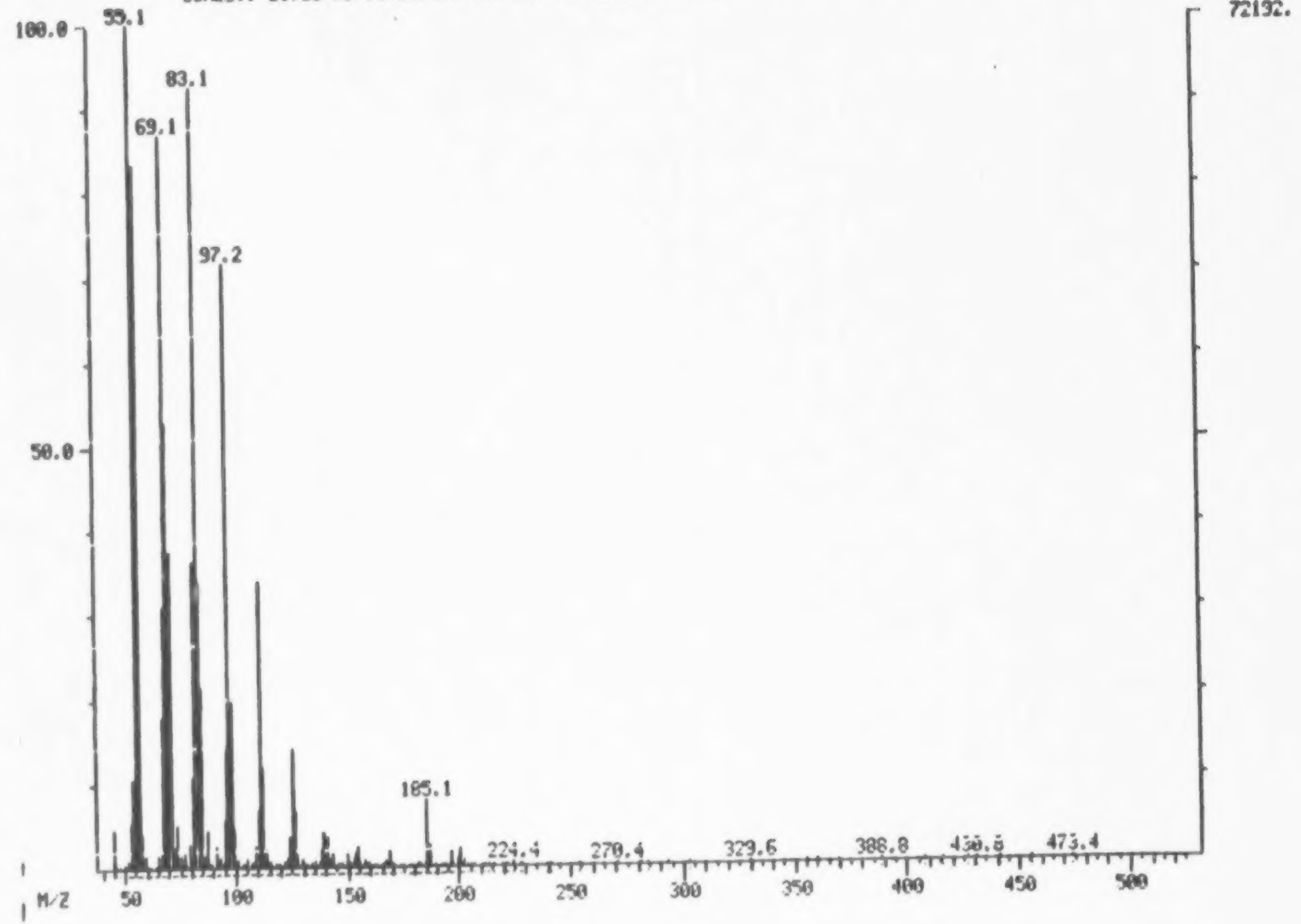


MM5 SAMPLE 2
PEAK 1
COMPARISON LIBRARY MASS SPECTRA

MASS SPECTRUM
05/06/86 16:28:00 + 19:06
SAMPLE: 41702-08-02 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

DATA: 567720 #1627
CALI: 567610CAL32 #4

BASE M/Z: 55
RIC: 705536.



MM5 SAMPLE 2
PEAK 8
MASS SPECTRUM

```
Library Search                   Data: 567720 #1627         Base m/z:    55
05/06/86 16:28:00 + 19:06        Cali: 567610CAL32 #   4    RIC:      666623.
Sample: 41702-08-02 MMS EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

39763 spectra in LIBRARYNB searched for maximum PURITY
  156 matched at least 7 of the 16 largest peaks in the unknown

Rank In.        Name
1 36956 1-DOTRIACONTANOL
2 22991 5-OCTADECENE, (E)-
3 22992 9-OCTADECENE, (E)-
4 25986 5-EICOSENE, (E)-
5 23495 ISOHEPTADECANOL
6 25985 9-EICOSENE, (E)-
7 19558 1-HEXADECENE
8 19557 CYCLOHEXADECANE
9 15402 CYCLOTETRADECANE

Rank   Formula                                     M.Wt B.Pk    Purity    Fit    RFit
1      C32.H66.O                                    466   57       737    921     786
2      C18.H36                                      252   55       721    895     799
3      C18.H36                                      252   55       714    887     798
4      C20.H40                                      280   55       714    903     785
5      C17.H36.O                                    256   83       710    874     772
6      C20.H40                                      280   57       707    895     784
7      C16.H32                                      224   57       706    897     781
8      C16.H32                                      224   55       704    887     783
9      C14.H28                                      196   55       704    893     777

Rank      Ret Time       B.P.Int.       US.Par.1      US.Par.2        C.A.S. #
1                                         0.2480                       6624-79-9
2          ____           ____            0.5070        ____           7206-21-5
3          ____           ____            0.5760        ____           7206-25-9
4          ____           ____            0.5200        ____          74685-30-6
5          ____           ____            0.7280        ____          57289-07-3
6          ____           ____            0.5720        ____          74685-29-3
7          ____           ____            0.6730        ____            629-73-2
8          ____           ____            0.6180        ____            295-65-8
9          ____           ____            0.5840        ____            295-17-0
           ____           ____                          ____
```

MMS SAMPLE ::
PEAK ::
LIBRARY SEARCH REPORT

```
LIBRARY SEARCH                                         DATA: 567720 #1627        BASE M/Z:  55
05/06/86 16:28:00 + 19:06                              CALI: 567610CAL32 #   4   RIC:   666623.
SAMPLE: 41702-08-02 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
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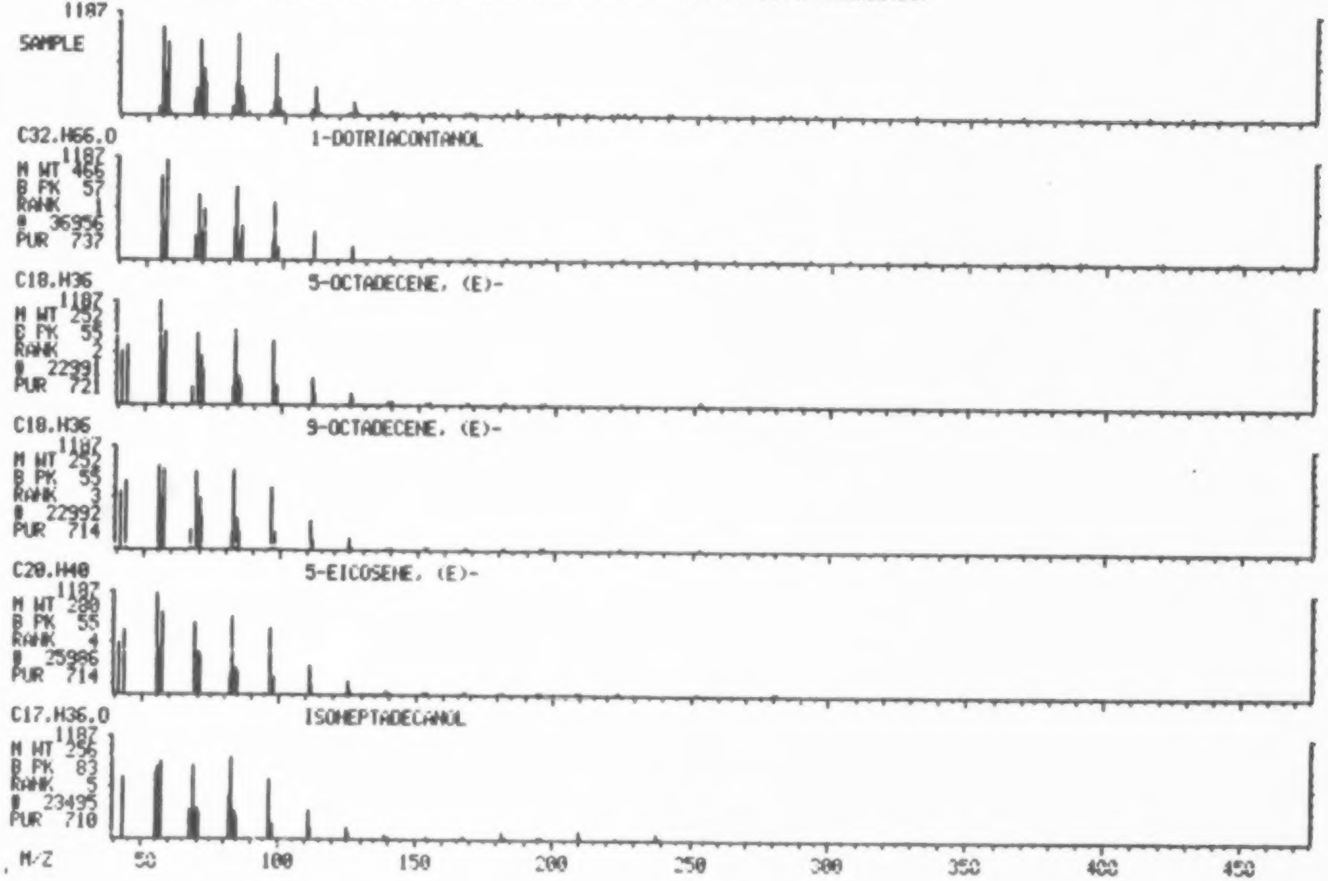

MM5 SAMPLE 2
PEAK 2
COMPARISON LIBRARY MASS SPECTRA

```
MASS SPECTRUM                                             DATA: 567720 #1645        BASE M/Z:  55
05/06/86 16:28:00 + 19:19                                 CALI: 567610CAL32 #4      RIC:    723968.
SAMPLE: 41702-08-02 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
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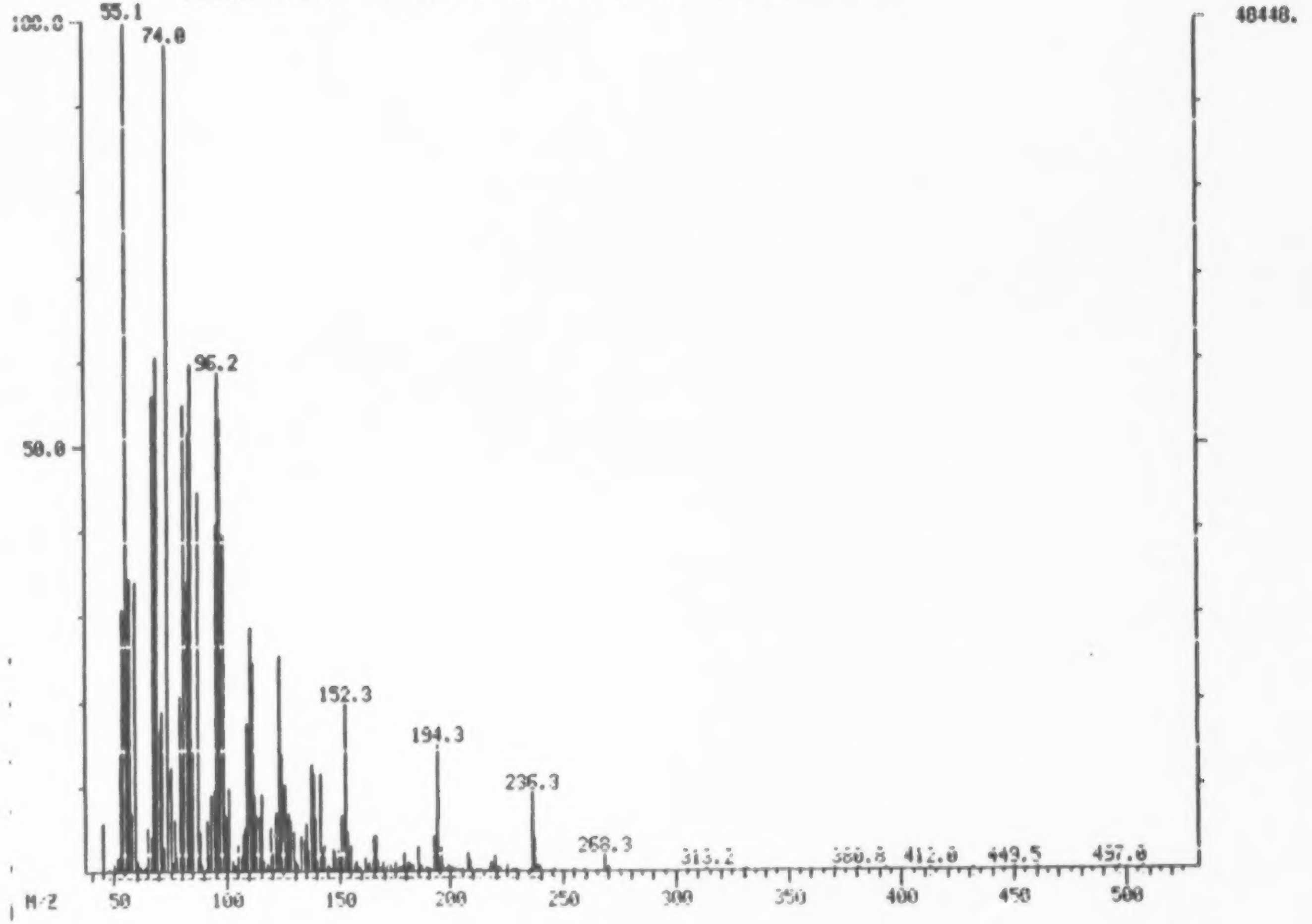

MM5 SAMPLE 2
PEAK 3
MASS SPECTRUM

```
Library Search                  Data: 567720 #1645          Base m/z:    55
05/06/86 16:28:00 + 19:19       Cali: 567610CAL32 #    4    RIC:      609279.
Sample: 41702-08-02 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI.GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

39763 spectra in LIBRARYNB searched for maximum PURITY
  205 matched at least 6 of the 16 largest peaks in the unknown

Rank In.        Name
1 24734 9-HEXADECENOIC ACID, METHYL ESTER, (Z)-
2 16233 10-UNDECENOYL CHLORIDE
3 23201 9-HEXADECENOIC ACID
4 30024 EICOSENOIC ACID, METHYL ESTER
5 25311 9-EICOSYNE
6 28996 ETHANOL, 2-(9-OCTADECENYLOXY)-, (Z)-
7 14980 1,13-TETRADECADIENE
8 28999 ETHANOL, 2-(9-OCTADECENYLOXY)-, (E)-
9 24764 9-OCTADECEN-1-OL, (Z)-

Rank  Formula                                   M.Wt B.Pk   Purity     Fit    RFit
1     C17.H32.O2                                 268   55      636     950     664
2     C11.H19.O.CL                               202   55      537     943     543
3     C16.H30.O2                                 254   55      517     851     581
4     C21.H40.O2                                 324   43      507     850     579
5     C20.H38                                    278   81      503     853     548
6     C20.H40.O2                                 312   55      503     850     552
7     C14.H26                                    194   55      488     879     518
8     C20.H40.O2                                 312   55      484     850     518
9     C18.H36.O                                  268   55      477     842     524

Rank      Ret.Time      B.P.Int       US.Par.1     US.Par.2       C.A.S. #
1          ____          ____         0.7130        ____          1120-25-8
2          ____          ____         0.3840        ____         38460-95-6
3          ____          ____         0.6330        ____          2091-29-4
4          ____          ____         0.6110        ____         27070-40-2
5          ____          ____         0.7130        ____         71899-38-2
6          ____          ____         0.6760        ____          5353-25-3
7          ____          ____         0.6100        ____         21964-49-8
8          ____          ____         0.7200        ____         17367-07-6
9          ____          ____         0.7120        ____           143-28-2
```

MM5 SAMPLE [illegible]
PEAK [illegible]
LIBRARY SEARCH REPORT

```
LIBRARY SEARCH                                          DATA: 567720 #1645        BASE M/Z:  55
05/06/86 16:28:00 + 19:19                               CALI: 567610CAL32 #   4   RIC:   609209.
SAMPLE: 41702-08-02 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
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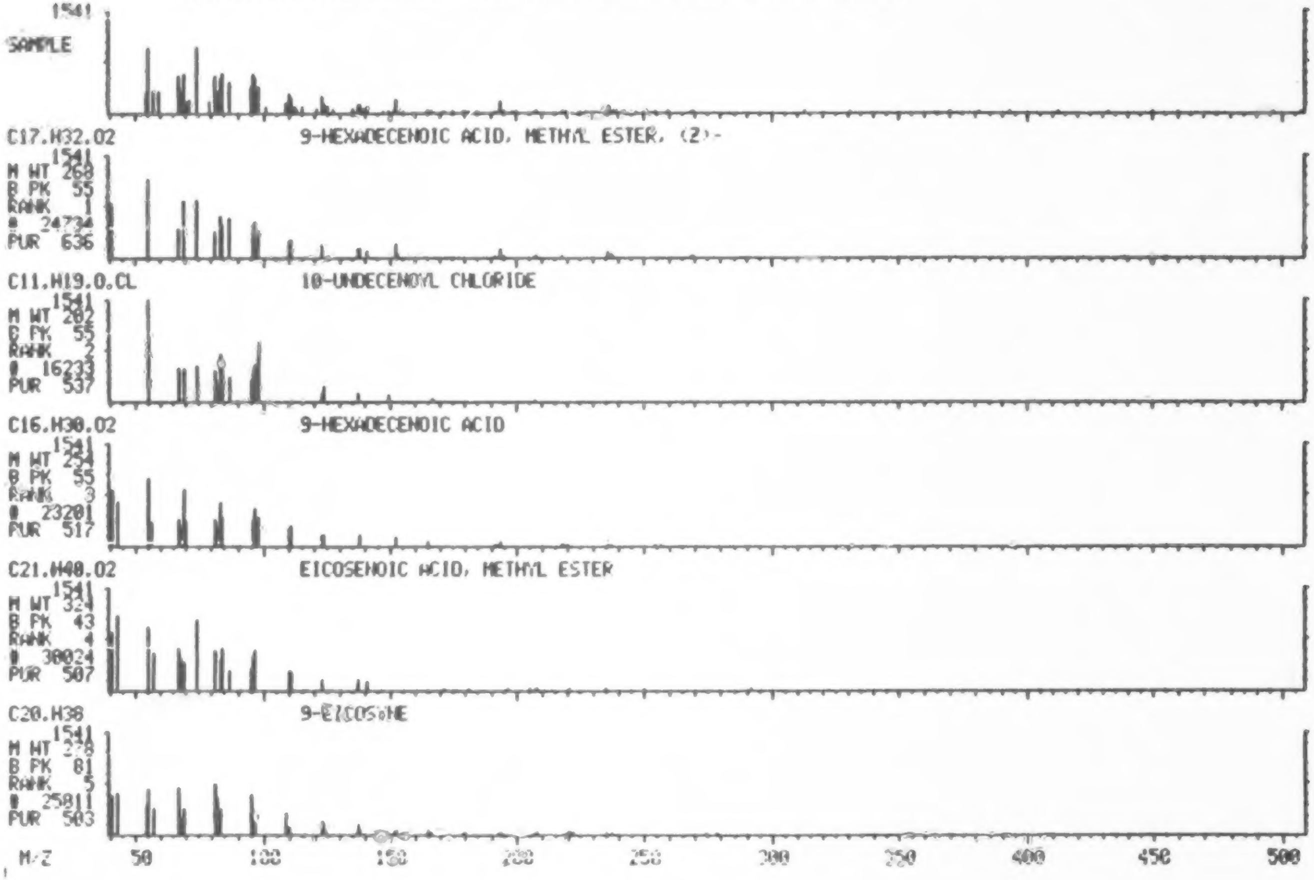
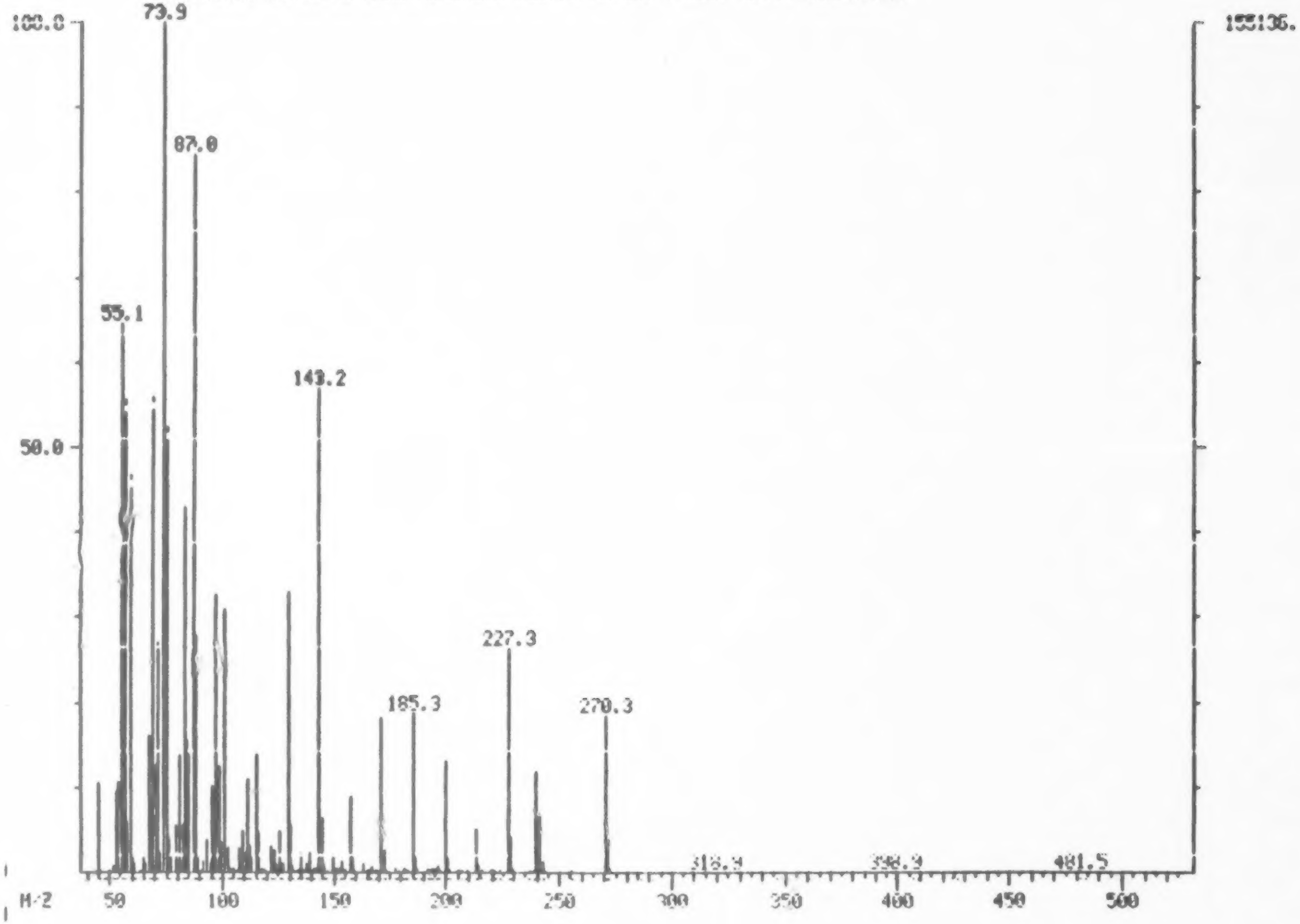


MM5 SAMPLE 2
PEAK 3
COMPARISON LIBRARY MASS SPECTRA

```
MASS SPECTRUM                                        DATA: 567720 #1665         BASE M/Z:  74
05/06/86 16:28:00 + 19:33                            CALI: 567610CAL32 #4       RIC:  1785850.
SAMPLE: 41702-08-02 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI.GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
```



MM5 SAMPLE 2
PEAK 4
MASS SPECTRUM

```
Library Search                Data: 567720 #1665        Base m/z:   74
05/06/86 16:28:00 + 19:33     Cali: 567610CAL32 #   4   RIC:    1609720.
Sample: 41702-08-02 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

39763 spectra in LIBRARYNB searched for maximum PURITY
  343 matched at least 5 of the 16 largest peaks in the unknown

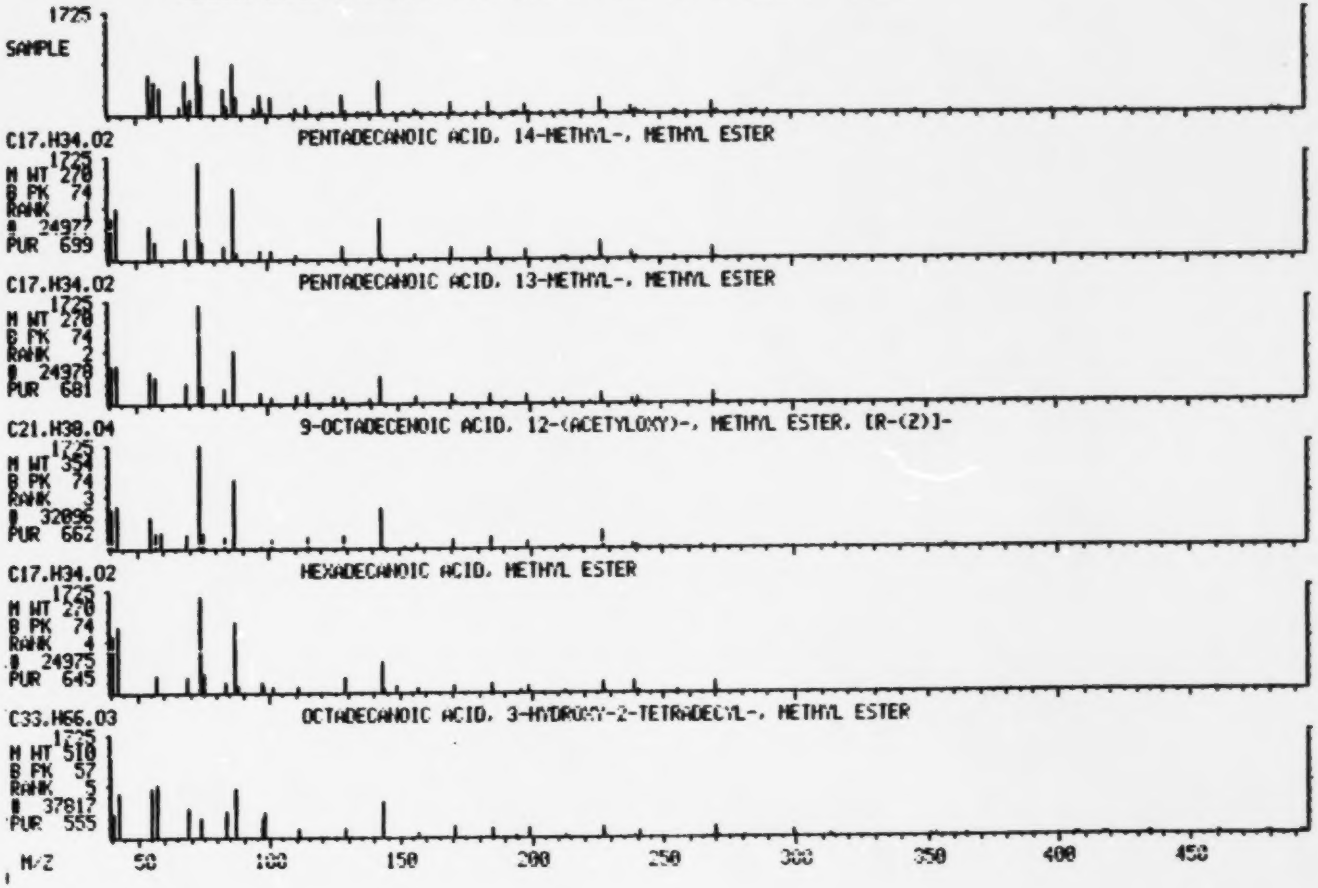
Rank In.        Name
1 24977 PENTADECANOIC ACID, 14-METHYL-, METHYL ESTER
2 24978 PENTADECANOIC ACID, 13-METHYL-, METHYL ESTER
3 32096 9-OCTADECENOIC ACID, 12-(ACETYLOXY)-, METHYL ESTER, [R-(Z)]-
4 24975 HEXADECANOIC ACID, METHYL ESTER
5 37317 OCTADECANOIC ACID, 3-HYDROXY-2-TETRADECYL-, METHYL ESTER
6 23478 TETRADECANOIC ACID, 12-METHYL-, METHYL ESTER
7 20119 DODECANOIC ACID, 10-METHYL-, METHYL ESTER
8 21308 TETRADECANOIC ACID, METHYL ESTER
9 26377 HEXADECANOIC ACID, 15-METHYL-, METHYL ESTER

Rank  Formula                                     M.Wt B.Pk   Purity    Fit   RFit
1     C17.H34 O2                                   270   74      699    888    759
2     C17.H34.O2                                   270   74      681    873    739
3     C21 H38 O4                                   354   74      662    851    728
4     C17.H34 O2                                   270   74      645    843    719
5     C33 H66 O3                                   510   57      555    797    659
6     C16 H32 O2                                   256   74      510    775    576
7     C14 H28 O2                                   228   74      499    791    562
8     C15.H30.O2                                   242   74      496    772    586
9     C18 H36 O2                                   284   74      493    684    623

Rank      Ret.Time       B.P.Int         US.Par.1      US.Par.2        C.A.S. #
1                                          0.7090                     5129-60-2
           ____           ____                          ____
2                                          0.6430                     5437-50-3
           ____           ____                          ____
3                                          0.5950                      140-03-4
           ____           ____                          ____
4                                          0.7140                      112-39-0
           ____           ____                          ____
5                                          0.6910                    17369-87-8
           ____           ____                          ____
6                                          0.6840                     5129-66-8
           ____           ____                          ____
7                                          0.6300                     5129-65-7
           ____           ____                          ____
8                                          0.6440                      124-10-7
           ____           ____                          ____
9                                          0.6650                     6929-04-0
           ____           ____                          ____
```

MM5 SAMPLE 2
PEAK 4
LIBRARY SEARCH REPORT

```
LIBRARY SEARCH                                    DATA: 567720 #1665          BASE M/Z:  74
05/06/86 16:28:00 + 19:33                         CALI: 567610CAL32 #   4     RIC:  1609720.
SAMPLE: 41702-08-02 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
```

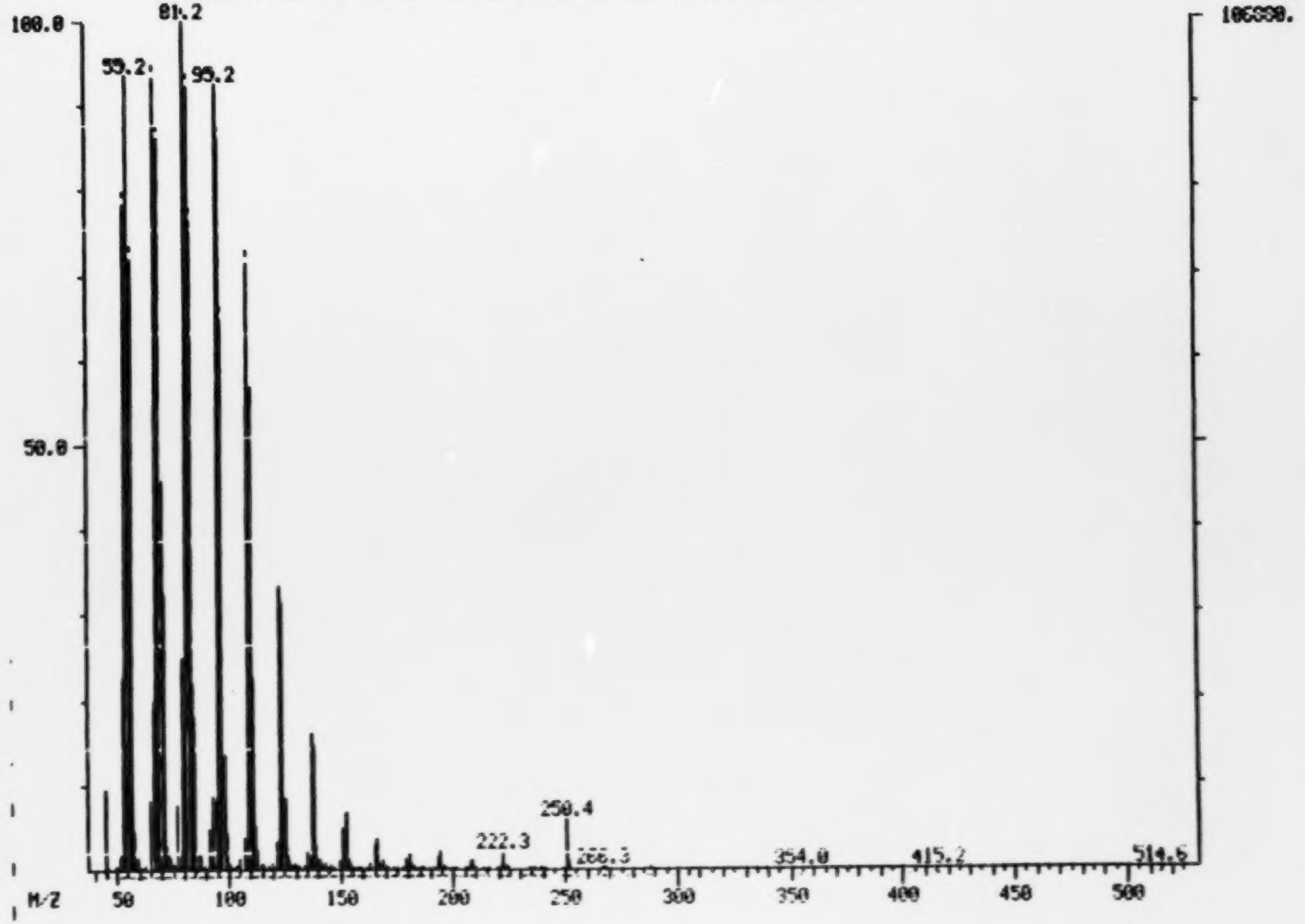


MM5 SAMPLE 2
PEAK 4
COMPARISON LIBRARY MASS SPECTRA

MASS SPECTRUM
05/06/86 16:28:00 + 20:49
SAMPLE: 41702-08-02 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 038779 HE(30) I(300) 60(4)-320@12(20)

DATA: 567720 #1772
CALI: 567610CAL32 #4

BASE M/Z: 81
RIC: 1812470.



MM5 SAMPLE 2
PEAK 5
MASS SPECTRUM

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Library Search                    Data: 567720 #1772          Base m/z:    81
05/06/86 16:28:00 + 20:49         Cali: 567610CAL32 #    4    RIC:    1705980.
Sample: 41702-08-02 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

39763 spectra in LIBRARYNB searched for maximum PURITY
  266 matched at least 7 of the 16 largest peaks in the unknown

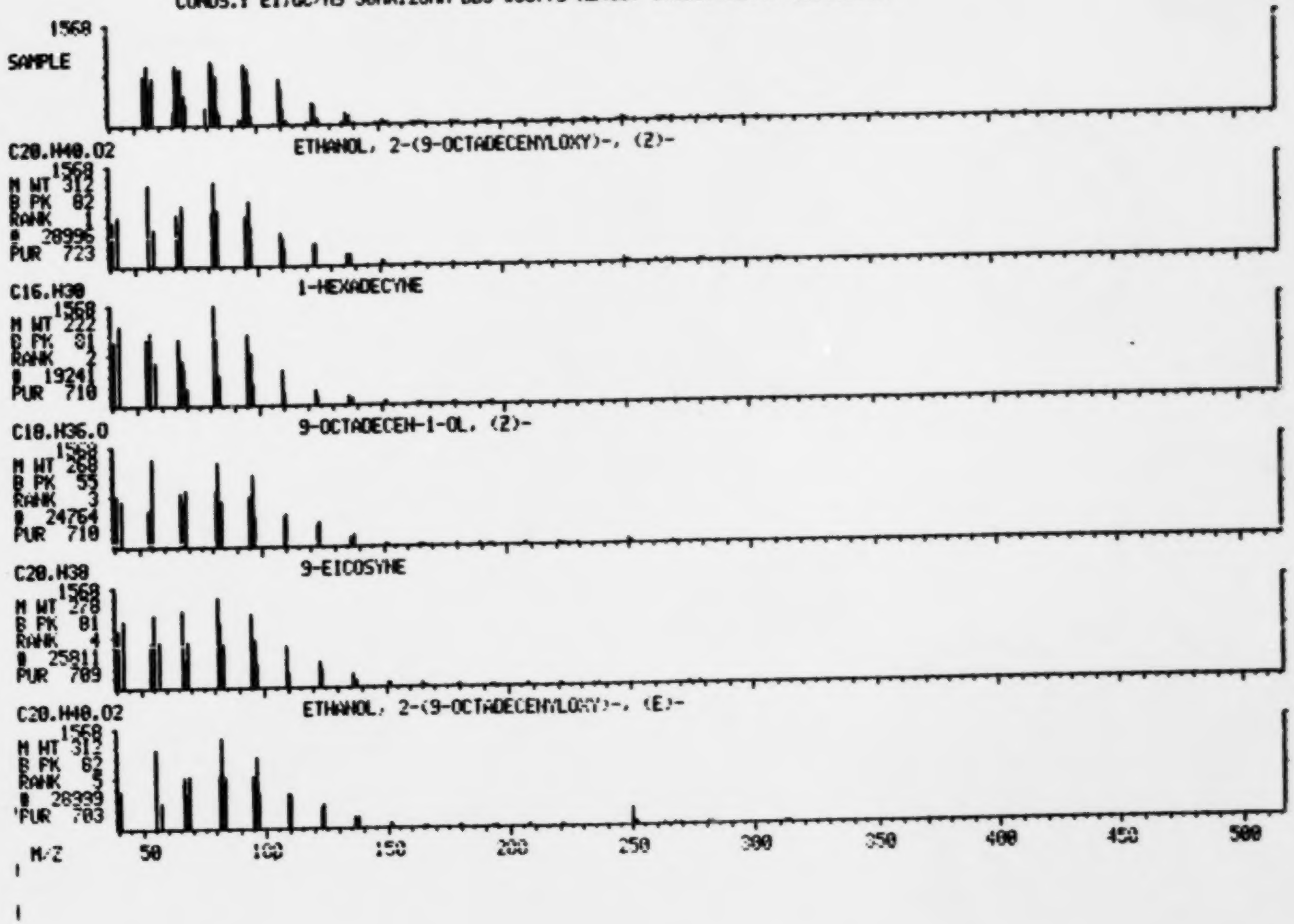
Rank In.       Name
1 28996 ETHANOL, 2-(9-OCTADECENYLOXY)-, (Z)-
2 19241 1-HEXADECYNE
3 24764 9-OCTADECEN-1-OL, (Z)-
4 25811 9-EICOSYNE
5 28999 ETHANOL, 2-(9-OCTADECENYLOXY)-, (E)-
6 14980 1,13-TETRADECADIENE
7 21590 HEXADECANAL
8 16266 1,12-DODECANEDIOL
9 25810 3-EICOSYNE

Rank  Formula                          M.Wt  B.Pk   Purity   Fit   RFit
1     C20.H40.O2                        312   82     723     924   772
2     C16.H30                           222   81     710     881   777
3     C18.H36.O                         268   55     710     927   756
4     C20.H38                           278   81     709     904   765
5     C20.H40 O2                        312   82     703     917   735
6     C14.H26                           194   55     698     939   731
7     C16.H32 O                         240   82     687     883   741
8     C12.H26.O2                        202   55     681     912   725
9     C20.H38                           278   67     679     877   700

Rank    Ret.Time    B P.Int.    US.Par 1    US.Par.2     C.A.S. #
1        ____        ____        0.6760      ____       5353-25-3
2        ____        ____        0.4570      ____        629-74-3
3        ____        ____        0.7120      ____        143-28-2
4        ____        ____        0.7130      ____      71899-38-2
5        ____        ____        0.7200      ____      17367-07-6
6        ____        ____        0.6100      ____      21964-49-8
7        ____        ____        0.6550      ____        629-80-1
8        ____        ____        0.6910      ____       5675-51-4
9        ____        ____        0.6960      ____      61886-66-6
```

MM5 SAMPLE 3
PEAK 5
LIBRARY SEARCH REPORT

LIBRARY SEARCH DATA: 567720 #1772 BASE M/Z: 81
05/06/86 16:28:00 + 20:49 CALI: 567610CAL32 # 4 RIC: 1705900.
SAMPLE: 41702-08-02 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

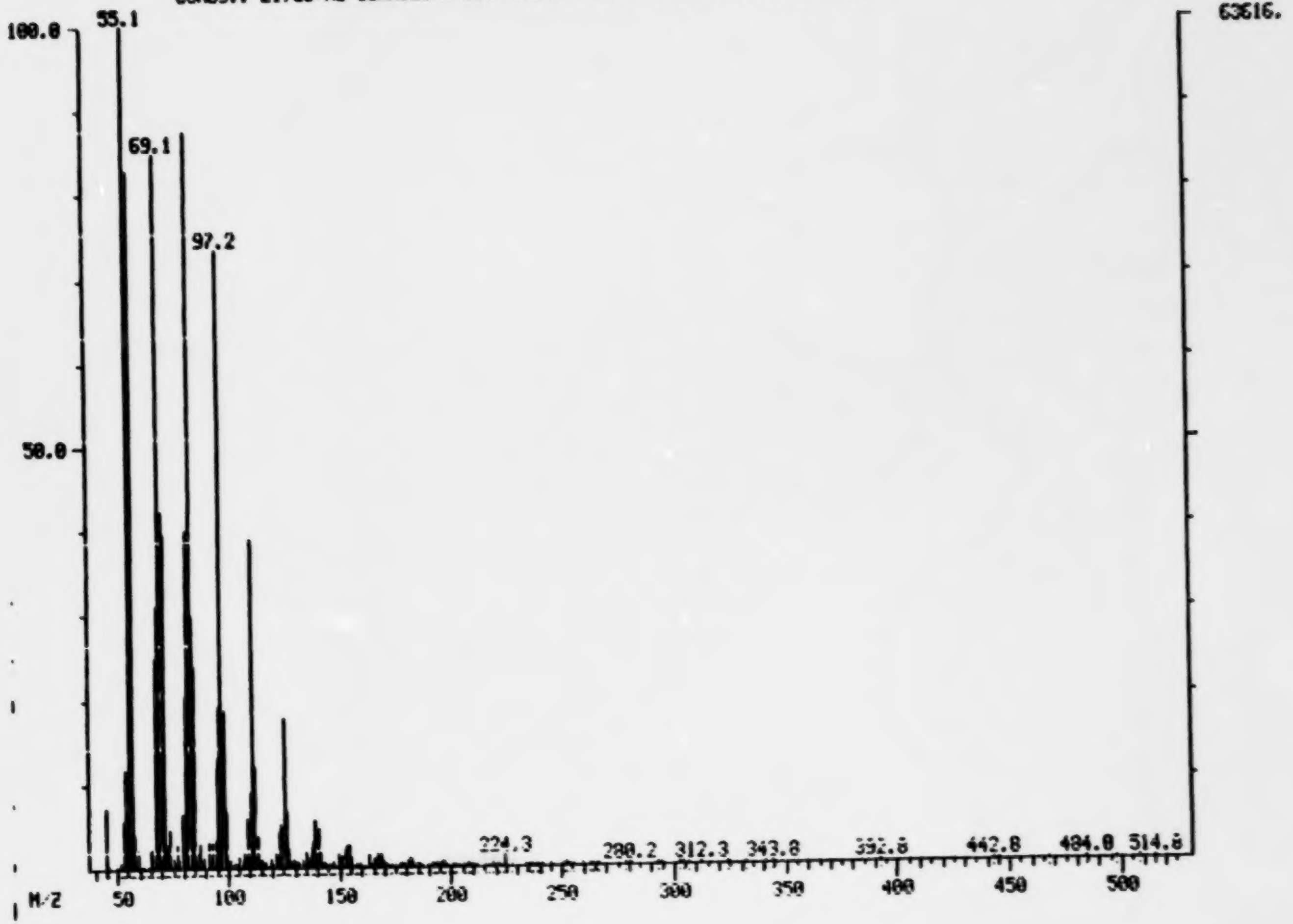


MM5 SAMPLE 2
PEAK 5
COMPARISON LIBRARY MASS SPECTRA

MASS SPECTRUM
05/06/86 16:28:00 + 20:58
SAMPLE: 41702-08-02 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

DATA: 567720 #1785
CALI: 567610CAL32 #4

BASE M/Z: 55
RIC: 660480.



MM5 SAMPLE 2
PEAK 6
MASS SPECTRUM

```
Library Search                 Data: 567720 #1785          Base m/z:    55
05/06/86 16.28:00 + 20:58      Cali: 567610CAL32 #    4    RIC:     610303.
Sample: 41702-08-02 MMS EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

39763 spectra in LIBRARYNB searched for maximum PURITY
  174 matched at least 7 of the 16 largest peaks in the unknown

Rank In.        Name
1 36956 1-DOTRIACONTANOL
2 22991 5-OCTADECENE, (E)-
3 23495 ISOHEPTADECANOL
4 22992 9-OCTADECENE, (E)-
5 25986 5-EICOSENE, (E)-
6 25985 9-EICOSENE, (E)-
7 24541 5-OCTADECENAL
8 19557 CYCLOHEXADECANE
9 21937 HEXADECANOL (VANBCI9CI)

Rank   Formula                                        M.Wt  B.Pk    Purity    Fit    RFit
1      C32.H66.O                                      466    57       707     936     748
2      C18.H36                                        252    55       691     905     751
3      C17.H36.O                                      256    83       685     901     740
4      C18.H36                                        252    55       683     895     751
5      C20.H40                                        280    55       682     912     742
6      C20.H40                                        280    57       673     901     739
7      C18.H34.O                                      266    57       673     868     732
8      C16.H32                                        224    55       669     900     739
9      C16.H34.O                                      242    43       665     895     735

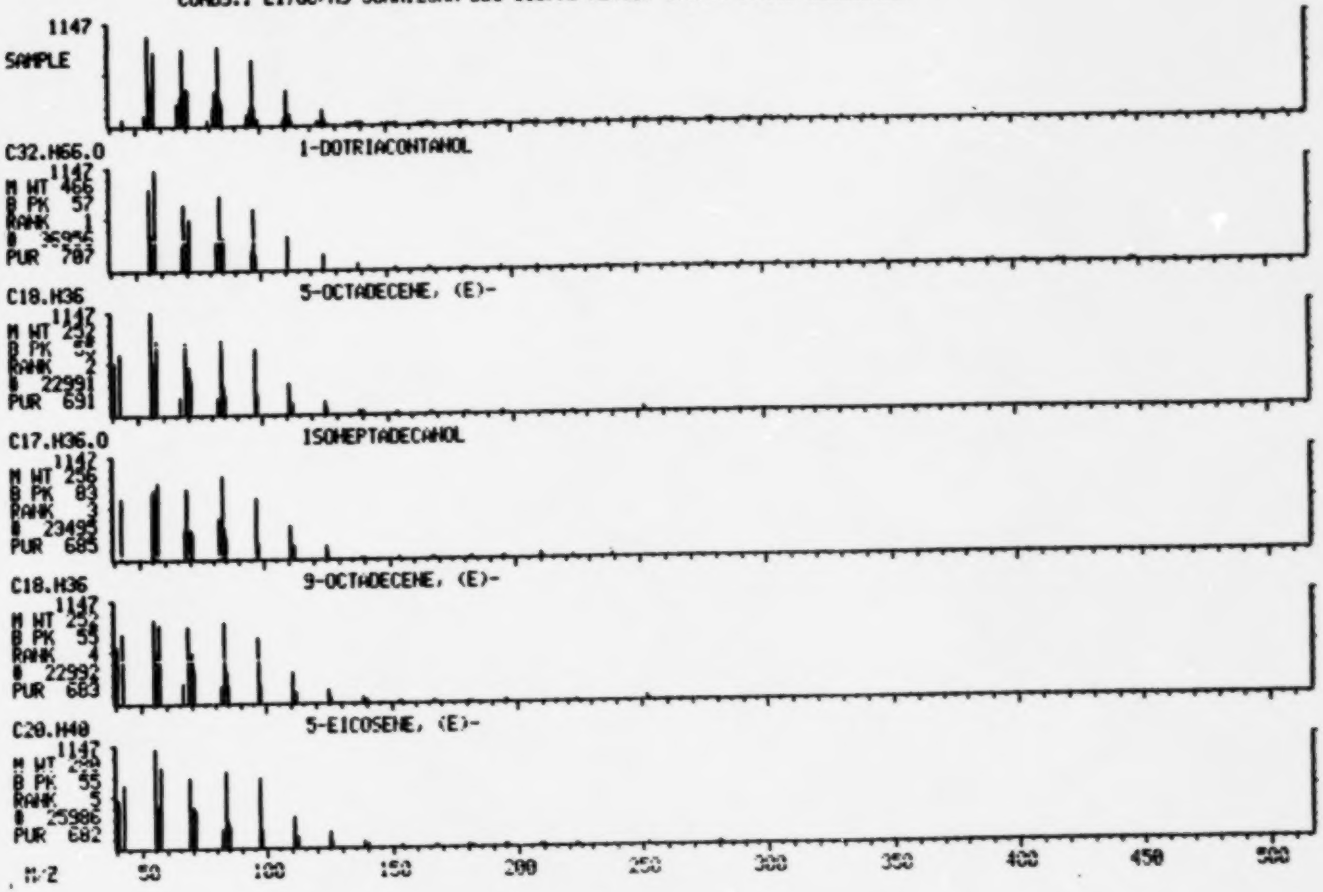
Rank      Ret.Time       B.P.Int.       US.Par.1       US.Par.2        C.A.S. #
1           ----           ----          0.2480           ----          6624-79-9
2           ----           ----          0.5070           ----          7206-21-5
3           ----           ----          0.7280           ----         57289-07-3
4           ----           ----          0.5760           ----          7206-25-9
5           ----           ----          0.5200           ----         74685-30-6
6           ----           ----          0.5720           ----         74685-29-3
7           ----           ----          0.1140           ----         56554-88-2
8           ----           ----          0.6180           ----           295-65-8
9           ----           ----          0.6220           ----         29354-98-1
```

MMS SAMPLE 2
PEAK 6
LIBRARY SEARCH REPORT

LIBRARY SEARCH
05/06/86 16:28:00 + 20:58
SAMPLE: 41702-08-02 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

DATA: 567720 #1785
CALI: 567610CAL32 # 4

BASE M/Z: 55
RIC: 610303.

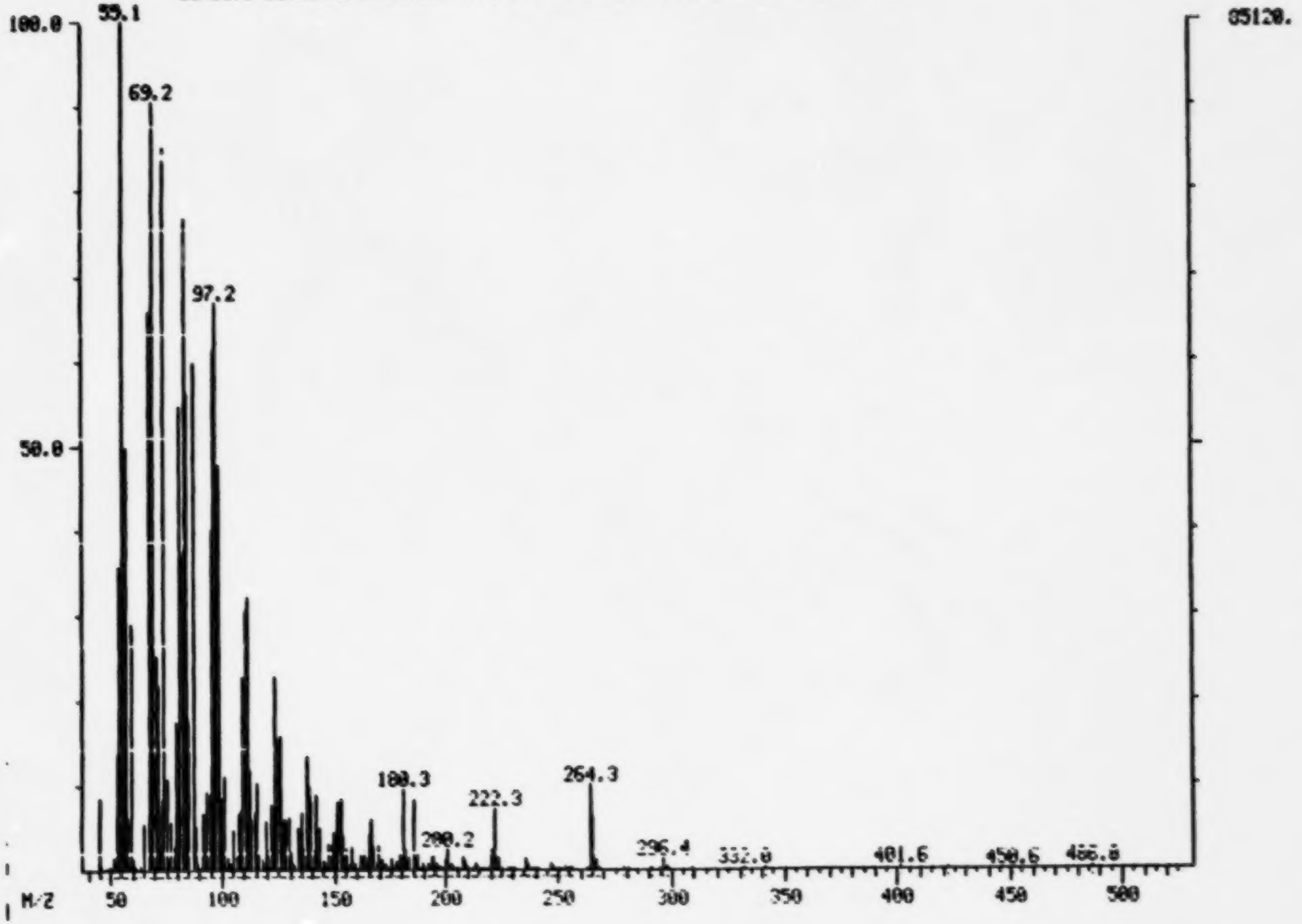


MM5 SAMPLE 2
PEAK 6
COMPARISON LIBRARY MASS SPECTRA

MASS SPECTRUM
05/06/86 16:28:00 + 21:08
SAMPLE: 41702-08-02 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

DATA: 567720 #1799
CALI: 567610CAL32 #4

BASE M/Z: 55
RIC: 1439740.



MMS SAMPLE 2
PEAK 7
MASS SPECTRUM

```
Library Search                     Data: 567720 #1799           Base m/z:    55
05/06/86 16:28:00 + 21:08          Cali: 567610CAL32 #    4     RIC:    1236990.
Sample: 41702-08-02 MMS EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

39763 spectra in LIBRARYNB searched for maximum PURITY
   503 matched at least 6 of the 16 largest peaks in the unknown

Rank In.       Name
1 27535 6-OCTADECENOIC ACID, METHYL ESTER
2 27525 10-OCTADECENOIC ACID, METHYL ESTER
3 27520 9-OCTADECENOIC ACID, METHYL ESTER
4 27519 8-OCTADECENOIC ACID, METHYL ESTER
5 27517 9-OCTADECENOIC ACID, METHYL ESTER, (E)-
6 27518 11-OCTADECENOIC ACID, METHYL ESTER, (Z)-
7 27538 12-OCTADECENOIC ACID, METHYL ESTER
8 27539 13-OCTADECENOIC ACID, METHYL ESTER
9 27540 14-OCTADECENOIC ACID, METHYL ESTER

Rank  Formula                                M.Wt B.Pk    Purity    Fit    RFit
1     C19.H36.O2                              296   55       670    991     670
2     C19.H36.O2                              296   55       607    930     649
3     C19.H36.O2                              296   55       595    924     641
4     C19.H36.O2                              296   55       592    920     641
5     C19.H36.O2                              296   55       592    922     640
6     C19.H36.O2                              296   55       579    936     617
7     C19.H36.O2                              296   55       574    940     608
8     C19.H36.O2                              296   55       568    942     599
9     C19.H36.O2                              296   55       559    939     591

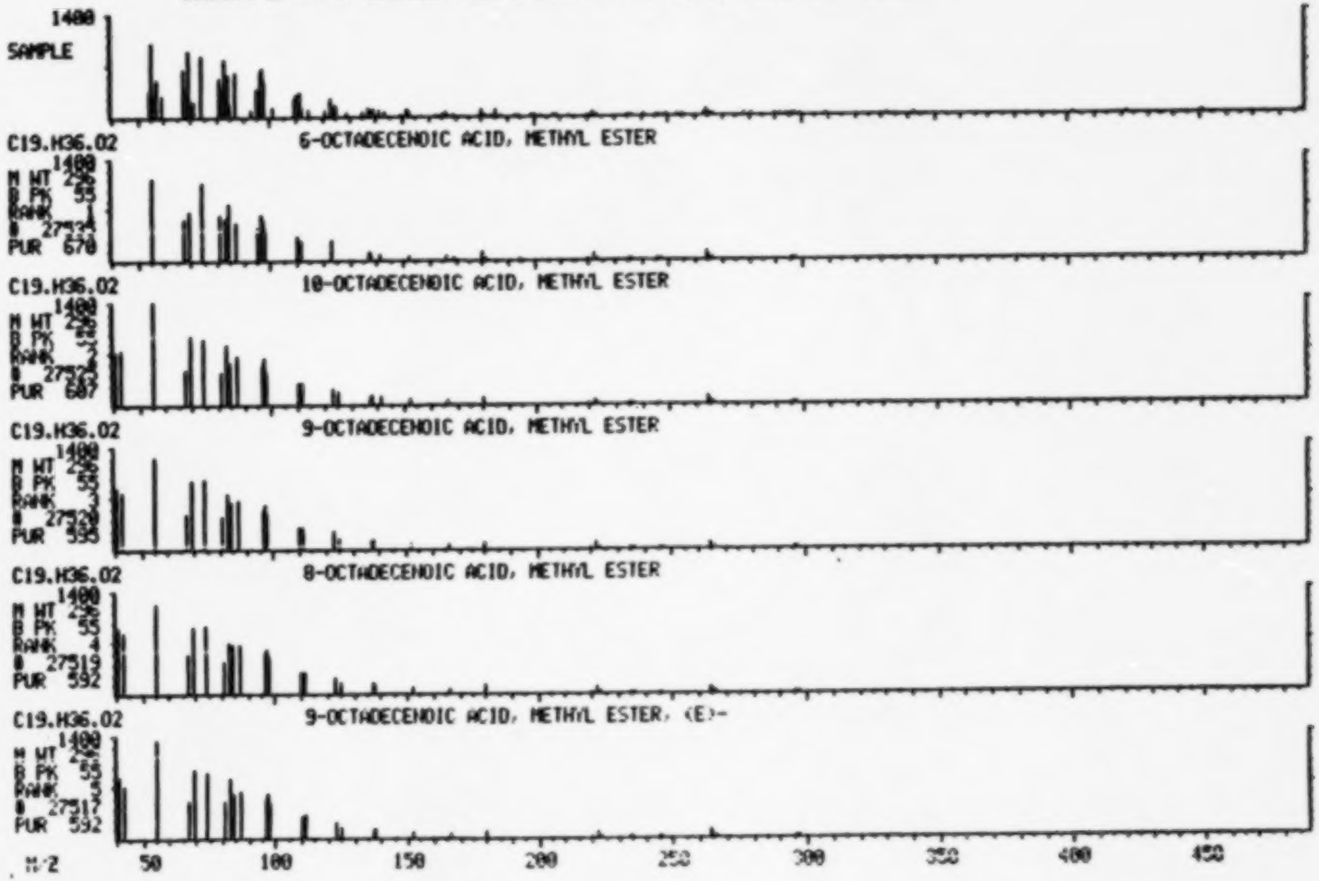
Rank      Ret.Time      B.P.Int.     US.Par.1      US.Par.2      C.A.S. #
1                                     0.7190                     52355-31-4
            ____          ____                       ____
2                                     0.6890                     13481-95-3
            ____          ____                       ____
3                                     0.7260                      2462-84-2
            ____          ____                       ____
4                                     0.7200                      2345-29-1
            ____          ____                       ____
5                                     0.7260                      1937-62-8
            ____          ____                       ____
6                                     0.7200                      1937-63-9
            ____          ____                       ____
7                                     0.7180                     56554-46-2
            ____          ____                       ____
8                                     0.6580                     56554-47-3
            ____          ____                       ____
9                                     0.6740                     56554-48-4
            ____          ____                       ____
```

MMS SAMPLE 2
PEAK 7
LIBRARY SEARCH REPORT

LIBRARY SEARCH
05/06/86 16:28:00 + 21:00
SAMPLE: 41702-08-02 MMS EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 038779 HE(30) I(300) 60(4)-320@12(20)

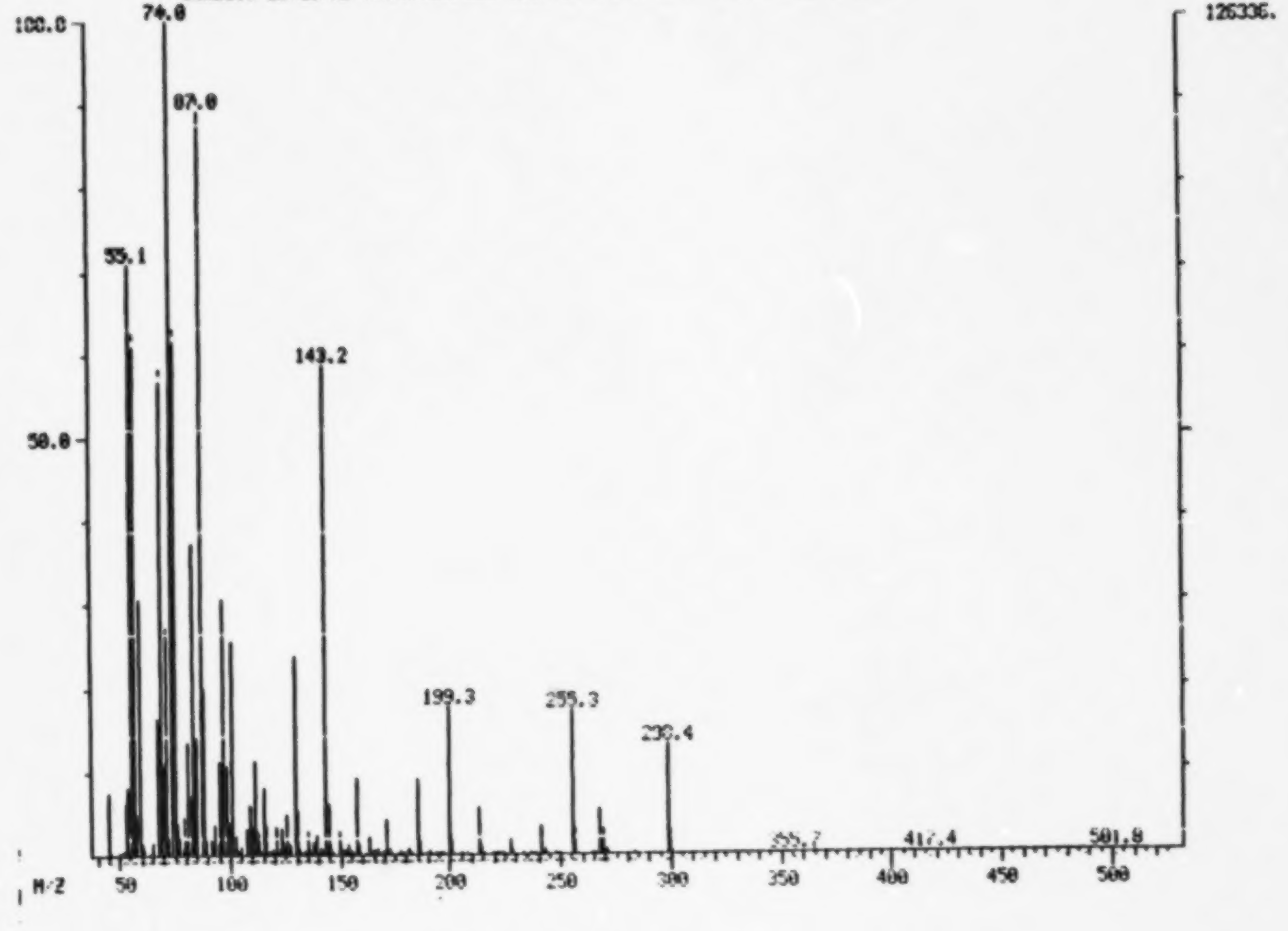
DATA: 567720 01799
CALI: 567610CAL32 # 4

BASE M/Z: 55
RIC: 1236990.



MMS SAMPLE 3
PEAK 7
COMPARISON LIBRARY MASS SPECTRA

```
MASS SPECTRUM                                          DATA: 567720 #1817        BASE M/Z:  74
05/06/86 16:28:00 + 21:20                              CALI: 567610CAL32 #4      RIC:  1370110.
SAMPLE: 41702-08-02 MMS EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI.GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
```



MMS SAMPLE 2
PEAK 8
MASS SPECTRUM

```
Library Search                    Data: 567720 #1817         Base m/z:    74
05/06/86 16:28:00 + 21:20         Cali: 567610CAL32 #   4    RIC:     1239030.
Sample: 41702-08-02 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

39763 spectra in LIBRARYNB searched for maximum PURITY
  116 matched at least 6 of the 16 largest peaks in the unknown

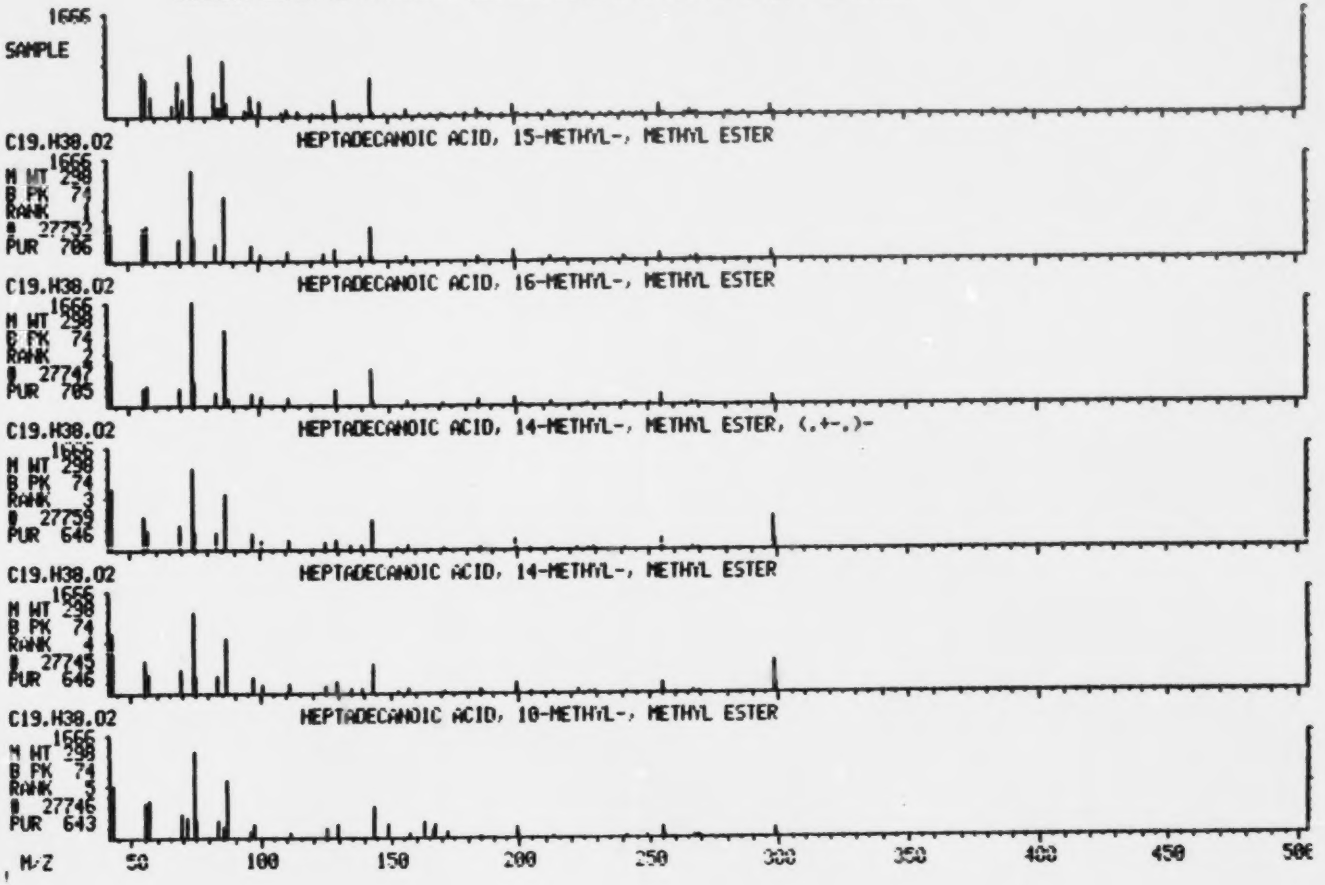
Rank In.       Name
1 27752 HEPTADECANOIC ACID, 15-METHYL-, METHYL ESTER
2 27747 HEPTADECANOIC ACID, 16-METHYL-, METHYL ESTER
3 27759 HEPTADECANOIC ACID, 14-METHYL-, METHYL ESTER, (.+-.)-
4 27745 HEPTADECANOIC ACID, 14-METHYL-, METHYL ESTER
5 27746 HEPTADECANOIC ACID, 10-METHYL-, METHYL ESTER
6 27754 HEPTADECANOIC ACID, 9-METHYL-, METHYL ESTER
7 27741 OCTADECANOIC ACID, METHYL ESTER
8 36952 TRIACONTANOIC ACID, METHYL ESTER
9 21308 TETRADECANOIC ACID, METHYL ESTER

Rank  Formula                               M.Wt  B.Pk    Purity    Fit    RFit
1     C19 H38 O2                             298   74       706     926     731
2     C19 H38 O2                             298   74       705     908     746
3     C19 H38 O2                             298   74       646     842     683
4     C19 H38 O2                             298   74       646     842     683
5     C19 H38 O2                             298   74       643     837     686
6     C19 H38 O2                             298   74       606     797     699
7     C19 H38 O2                             298   74       528     756     609
8     C31 H62 O2                             466   74       519     760     654
9     C15 H30 O2                             242   74       513     787     610

Rank     Ret.Time      B.P.Int.      US.Par.1     US.Par.2        C.A.S. #
1          ____          ____         0.7130        ____        54833-55-5
2          ____          ____         0.6960        ____         5129-61-3
3          ____          ____         0.7070        ____        57274-45-0
4          ____          ____         0.7110        ____         2490-23-5
5          ____          ____         0.6670        ____         2490-25-7
6          ____          ____         0.7140        ____        54934-57-5
7          ____          ____         0.7170        ____          112-61-8
8          ____          ____         0.3330        ____          629-83-4
9          ____          ____         0.6440        ____          124-10-7
```

MM5 SAMPLE 2
PEAK 8
LIBRARY SEARCH REPORT

LIBRARY SEARCH DATA: 567720 #1817 BASE M/Z: 74
05/06/86 16:28:00 + 21:20 CALI: 567610CAL32 # 4 RIC: 1239030.
SAMPLE: 41702-00-02 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI.GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

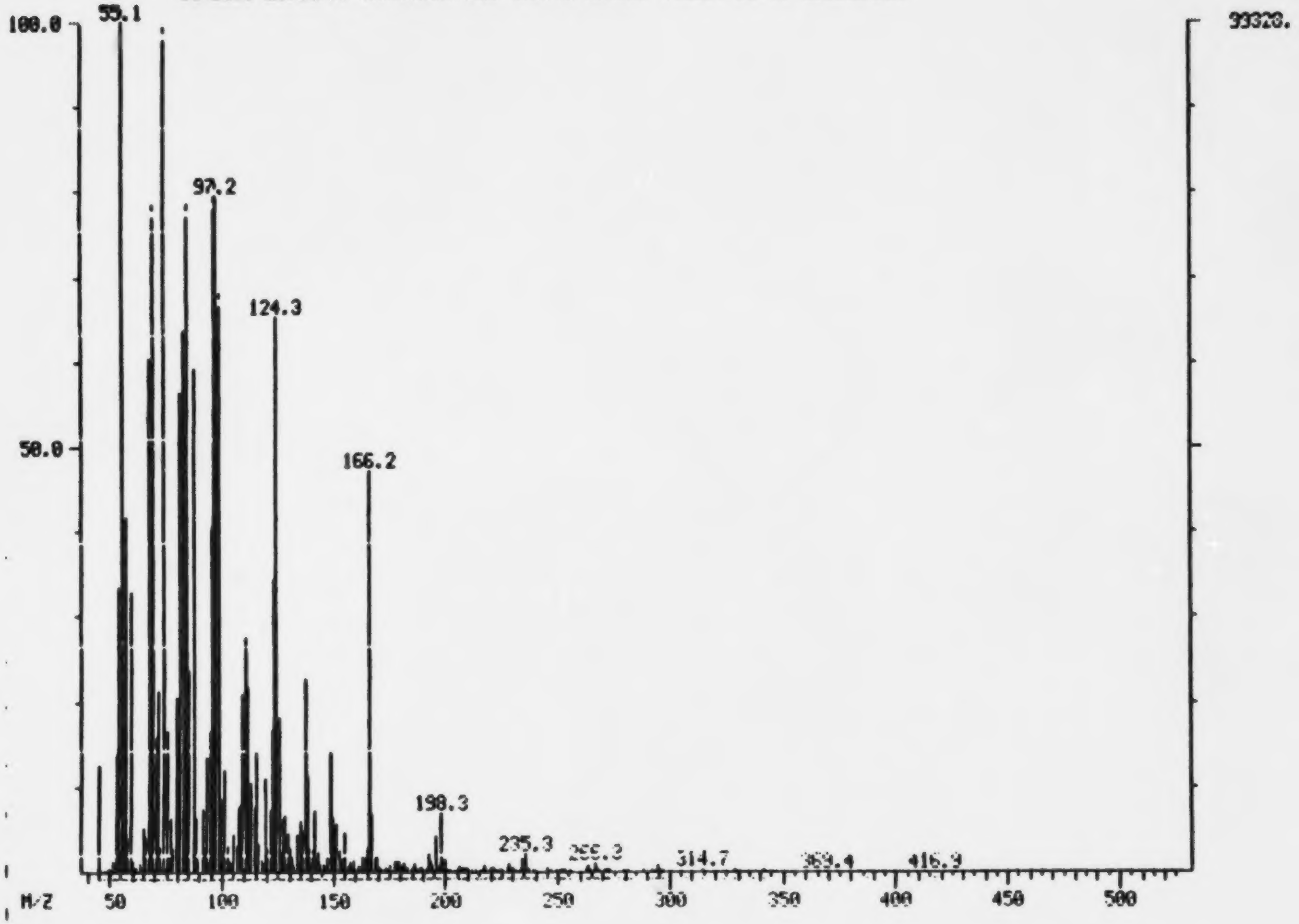


MM5 SAMPLE ::
PEAK 8
COMPARISON LIBRARY MASS SPECTRA

MASS SPECTRUM
05/06/86 16:28:00 + 22:46
SAMPLE: 41702-08-02 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

DATA: 567720 #1938
CALI: 567610CAL32 #4

BASE M/Z: 55
RIC: 1855480.



MM5 SAMPLE 2
PEAK 9
MASS SPECTRUM

```
Library Search                  Data: 567720 #1938          Base m/z:    55
05/06/86 16:28:00 + 22:46       Cali: 567610CAL32 #   4    RIC:    1556470.
Sample: 41702-08-02 MMS EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

39763 spectra in LIBRARYNB searched for maximum PURITY
  399 matched at least 6 of the 16 largest peaks in the unknown

Rank In.       Name
1 16233 10-UNDECENOYL CHLORIDE
2 27535 6-OCTADECENOIC ACID, METHYL ESTER
3 15642 10-UNDECENOIC ACID, METHYL ESTER
4 12773 CYCLOHEXANE, (2,2-DIMETHYLCYCLOPENTYL)-
5 10303 CYCLODODECENE, (E)-
6 28996 ETHANOL, 2-(9-OCTADECENYLOXY)-, (Z)-
7 14980 1,13-TETRADECADIENE
8 10302 CYCLODODECENE, (Z)-
9 13417 CYCLOHEXANEHEXANOL

Rank  Formula                                  M.Wt B.Pk   Purity   Fit   RFit
1     C11.H19.O.CL                              202   55      615   928    615
2     C19.H36.O2                                296   74      527   841    571
3     C12.H22.O2                                198   74      514   850    544
4     C13.H24                                   180   55      493   824    515
5     C12.H22                                   166   67      487   865    505
6     C20.H40.O2                                312   55      486   820    527
7     C14.H26                                   194   55      485   845    521
8     C12.H22                                   166   67      483   858    502
9     C12.H24.O                                 184   55      482   792    517

Rank     Ret.Time     B.P.Int.     US.Par.1     US.Par.2      C.A.S. #
1          ___          ___         0.8840        ___        38460-95-6
2          ___          ___         0.7190        ___        52355-31-4
3          ___          ___         0.7120        ___          111-81-9
4          ___          ___         0 6750        ___        61142-23-2
5          ___          ___         0.7130        ___         1486-75-5
6          ___          ___         0.6760        ___         5353-25-3
7          ___          ___         0.6100        ___        21964-49-8
8          ___          ___         0.6690        ___         1129-89-1
9          ___          ___         0.6910        ___         4354-58-9
```

MMS SAMPLE 2
PEAK 9
LIBRARY SEARCH REPORT

```
LIBRARY SEARCH                                   DATA: 567720 #1938        BASE M/Z:  55
05/06/86 16:28:00 + 22:46                        CALI: 567610CAL32 #   4   RIC:  1556470.
SAMPLE: 41702-08-02 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
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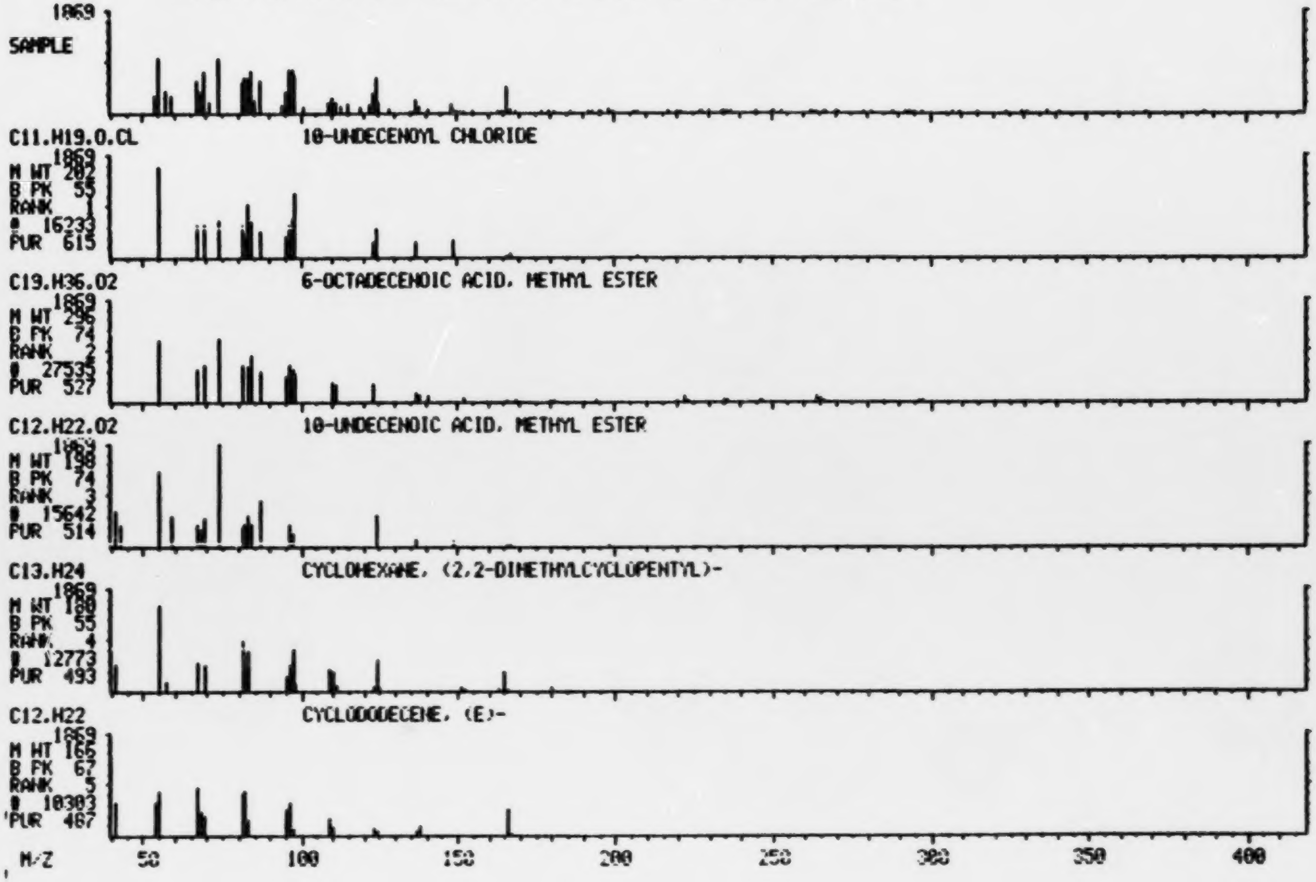
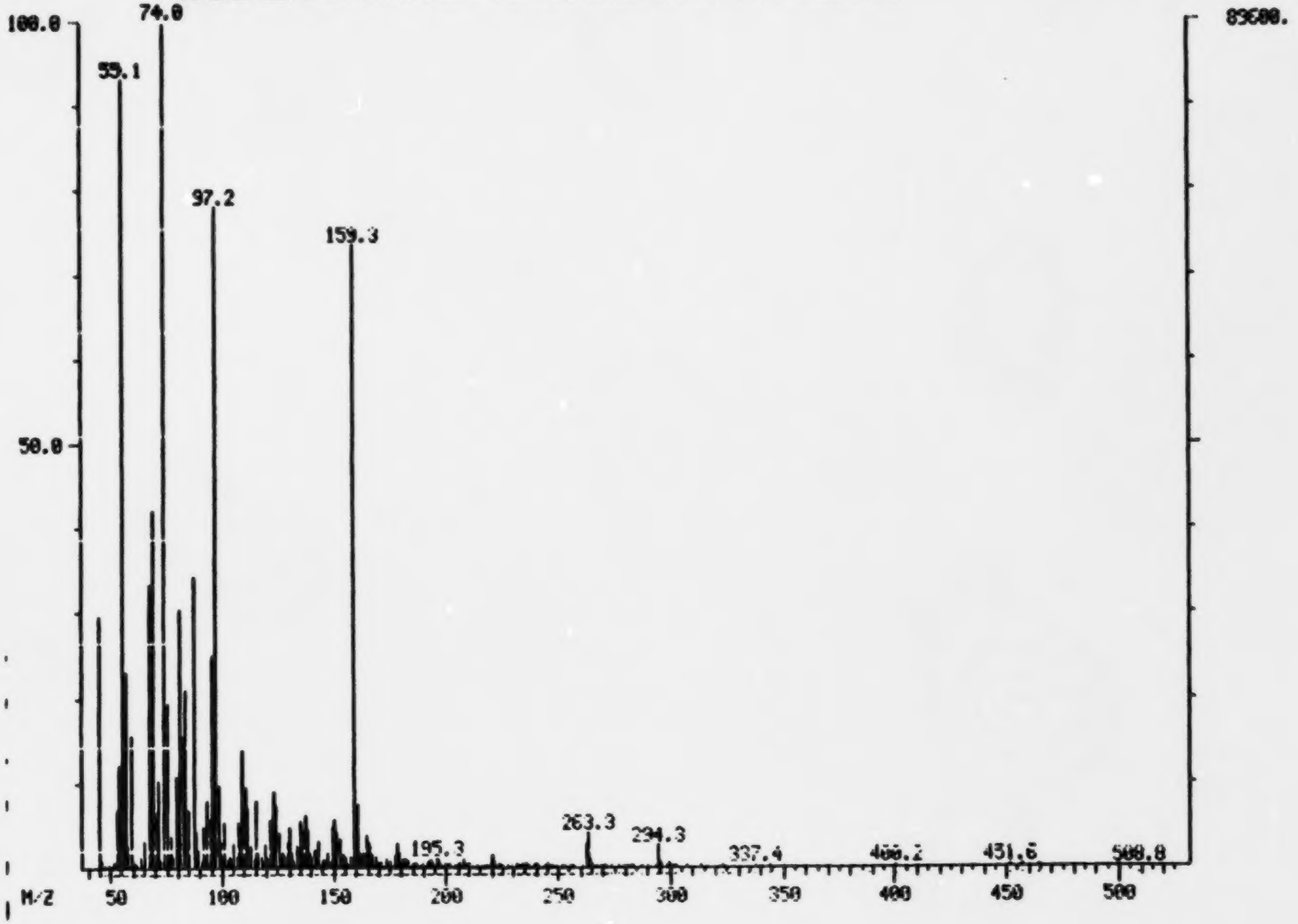


MM5 SAMPLE 2
PEAK 9
COMPARISON LIBRARY MASS SPECTRA

MASS SPECTRUM
05/06/86 16:28:00 + 24:22
SAMPLE: 41702-08-02 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

DATA: 567720 #2075
CALI: 567610CAL32 #4

BASE M/Z: 74
RIC: 932864.



MM5 SAMPLE 2
PEAK 10
MASS SPECTRUM

```
Library Search               Data: 567720 #2075          Base m/z:     74
05/06/86 16:28:00 + 24:22    Cali: 567610CAL32 #    4   RIC:      807935.
Sample: 41702-08-02 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

39763 spectra in LIBRARYNB searched for maximum PURITY
  379 matched at least 6 of the 16 largest peaks in the unknown

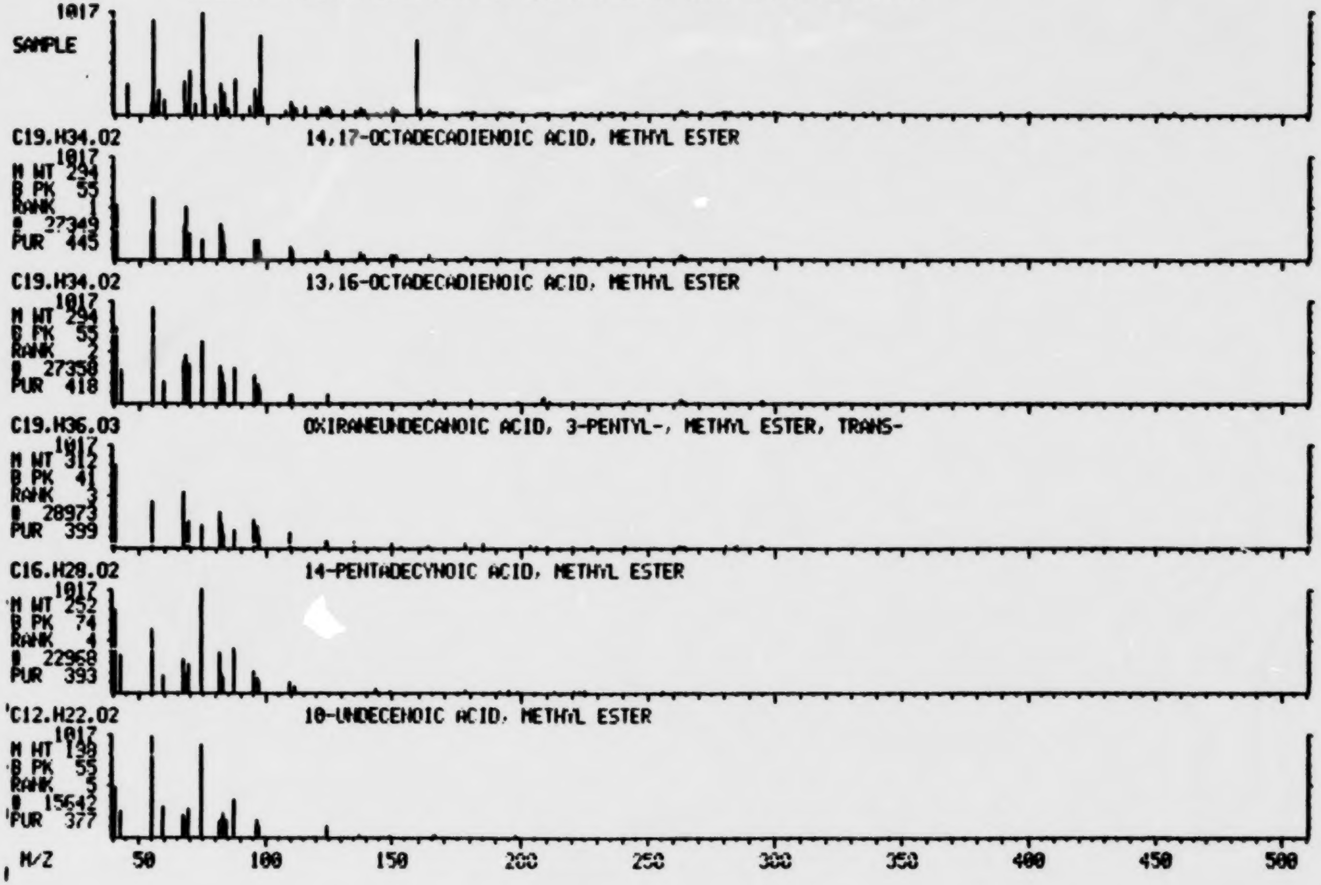
Rank In.       Name
1 27349 14,17-OCTADECADIENOIC ACID, METHYL ESTER
2 27358 13,16-OCTADECADIENOIC ACID, METHYL ESTER
3 28973 OXIRANEUNDECANOIC ACID, 3-PENTYL-, METHYL ESTER, TRANS-
4 22968 14-PENTADECYNOIC ACID, METHYL ESTER
5 15642 10-UNDECENOIC ACID, METHYL ESTER
6 37955 OCTADECANOIC ACID, 9,10-DIHYDROXY-, METHYL ESTER, BIS(TRIFLUOROACET*
7 12773 CYCLOHEXANE, (2,2-DIMETHYLCYCLOPENTYL)-
8 33555 15-TETRACOSENOIC ACID, METHYL ESTER, (Z)-
9 21303 13-TETRADECYNOIC ACID, METHYL ESTER

Rank   Formula                              M.Wt B.Pk   Purity    Fit   RFit
1      C19.H34.O2                            294   55      445    797    475
2      C19.H34.O2                            294   55      418    776    480
3      C19.H36.O3                            312   41      399    729    476
4      C16.H23.O2                            252   74      393    790    459
5      C12.H22.O2                            198   55      377    817    415
6      C23.H36.O6.F6                         522   74      374    806    453
7      C13.H24                               180   55      370    862    387
8      C25.H43.O2                            380   55      368    742    449
9      C15.H26.O2                            238   74      365    869    412

Rank     Ret.Time      B.P Int.      US.Par.1     US.Par.2      C.A.S. #
1          ___           ___          0.7160        ___        56554-60-0
2          ___           ___          0.7190        ___        56846-99-2
3          ___           ___          0.7160        ___        38520-31-9
4          ___           ___          0.1450        ___        56909-04-7
5          ___           ___          0.7120        ___          111-81-9
6          ___           ___          0.6360        ___        21987-19-9
7          ___           ___          0.6750        ___        61142-23-2
8          ___           ___          0.4510        ___         2733-88-2
9          ___           ___          0.7280        ___        56909-03-6
```

MM5 SAMPLE 2
PEAK 10
LIBRARY SEARCH REPORT

```
LIBRARY SEARCH                                       DATA: 567720 #2075      BASE M/Z:  74
05/06/86 16:28:00 + 24:22                            CALI: 567610CAL32 #  4  RIC:   807935.
SAMPLE: 41702-08-02 MM5 EXTRACT  2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
```



MM5 SAMPLE 2
PEAK 10
COMPARISON LIBRARY MASS SPECTRA

```
MASS SPECTRUM                                         DATA: 567814 #502          BASE M/Z: 100
05/07/86  9:54:00 +  5:54                             CALI: 567810CAL32 #4       RIC:    534528.
SAMPLE: 41702-08-03 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
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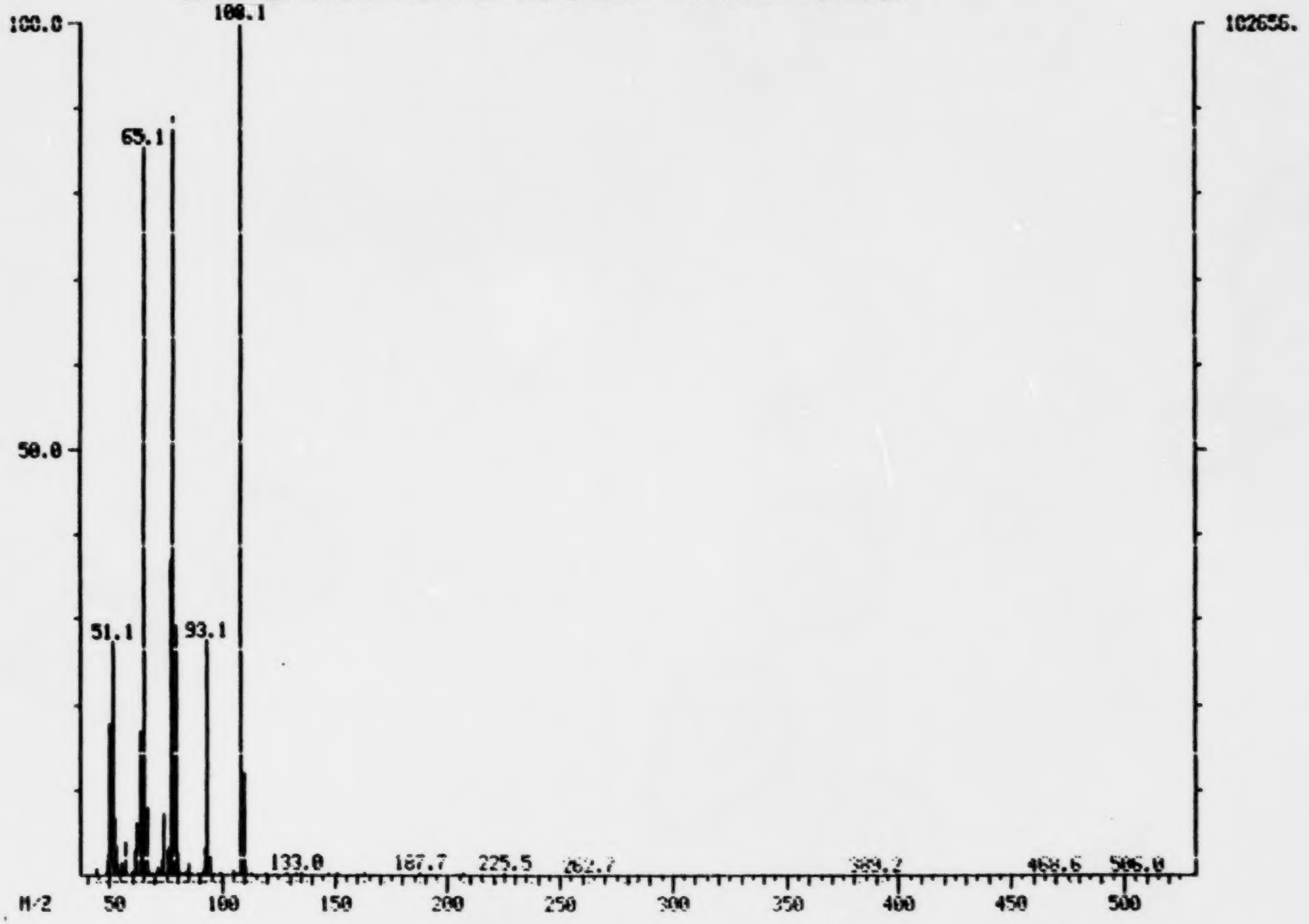


MM5 SAMPLE 3
PEAK 1
MASS SPECTRUM

```
Library Search                  Data: 567814 # 502         Base m/z:  108
05/07/86  9:54:00 +  5:54       Cali: 567810CAL32 #   4    RIC:     510463.
Sample: 41702-08-03 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

39763 spectra in LIBRARYNB searched for maximum PURITY
  353 matched at least 5 of the 16 largest peaks in the unknown

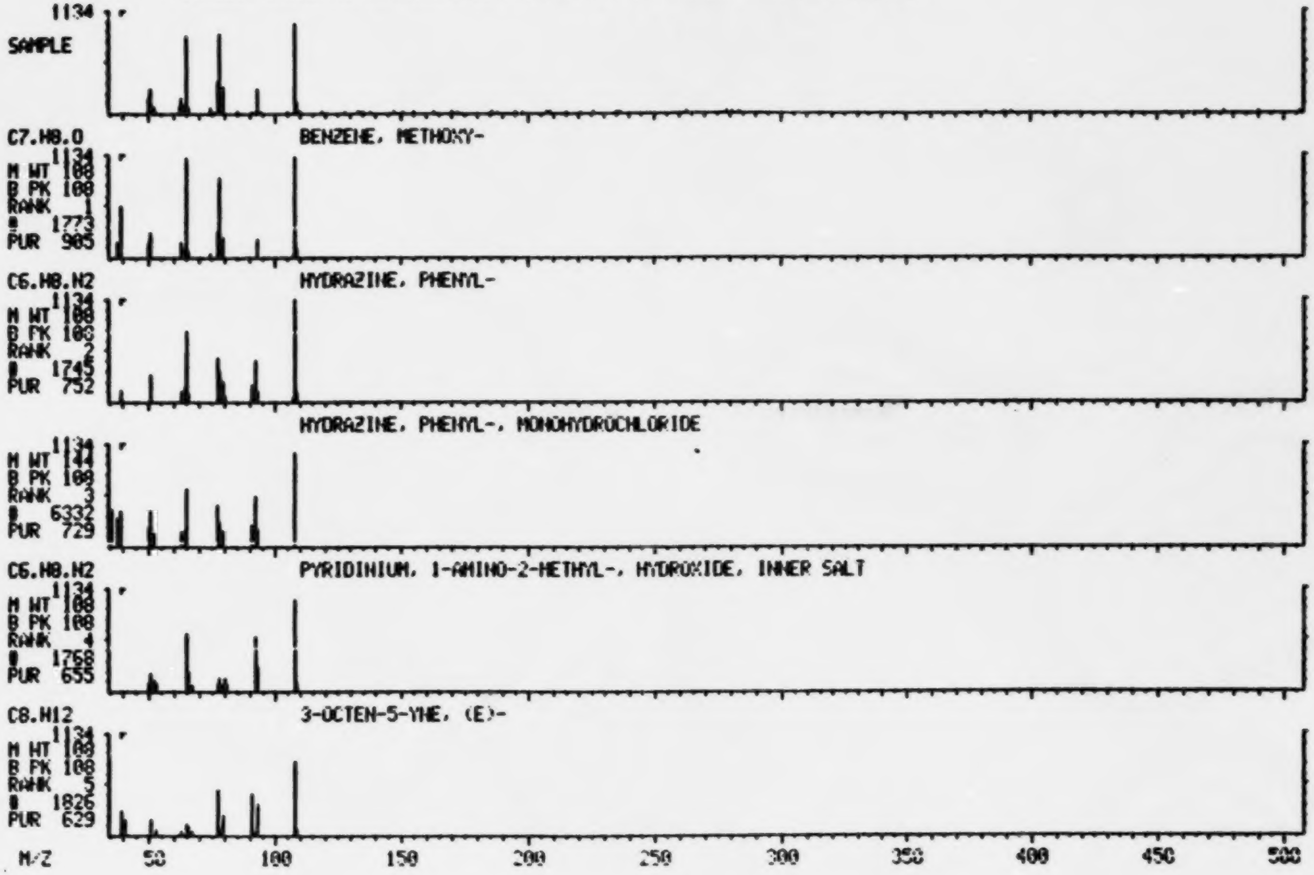
Rank In.        Name
1  1773 BENZENE, METHOXY-
2  1745 HYDRAZINE, PHENYL-
3  6332 HYDRAZINE, PHENYL-, MONOHYDROCHLORIDE
4  1768 PYRIDINIUM, 1-AMINO-2-METHYL-, HYDROXIDE, INNER SALT
5  1826 3-OCTEN-5-YNE, (E)-
6  1825 3-OCTEN-5-YNE, (Z)-
7  1821 2-HEPTEN-4-YNE, 2-METHYL-
8  1819 CYCLOHEXANE, 1,3-BIS(METHYLENE)-
9  3679 3,5-OCTADIEN-2-OL

Rank  Formula                                   M.Wt B.Pk   Purity    Fit   RFit
1     C7 H8 O                                    108  108      905    984    913
2     C6 H8 N2                                   108  108      752    841    776
3                                                144  108      729    829    729
4     C6 H8 N2                                   108  108      655    739    703
5     C8 H12                                     108  108      629    712    653
6     C8 H12                                     108  108      625    707    649
7     C8 H12                                     108  108      584    661    613
8     C8 H12                                     108   78      576    803    606
9     C8 H14 O                                   126  108      564    664    608

Rank      Ret.Time      B.P.Int      US.Par.1      US.Par.2      C.A.S. #
1          ___           ___          0.6520         ___          100-66-3
2          ___           ___          0.7270         ___          100-63-0
3          ___           ___          0.6910         ___           59-88-1
4          ___           ___          0.4900         ___         51135-75-2
5          ___           ___          0.7970         ___         74744-35-7
6          ___           ___          0.7980         ___         74744-34-6
7          ___           ___          0.7660         ___         58275-91-5
8          ___           ___          0.7690         ___         52086-82-5
9          ___           ___         ___             ___         69668-82-2
```

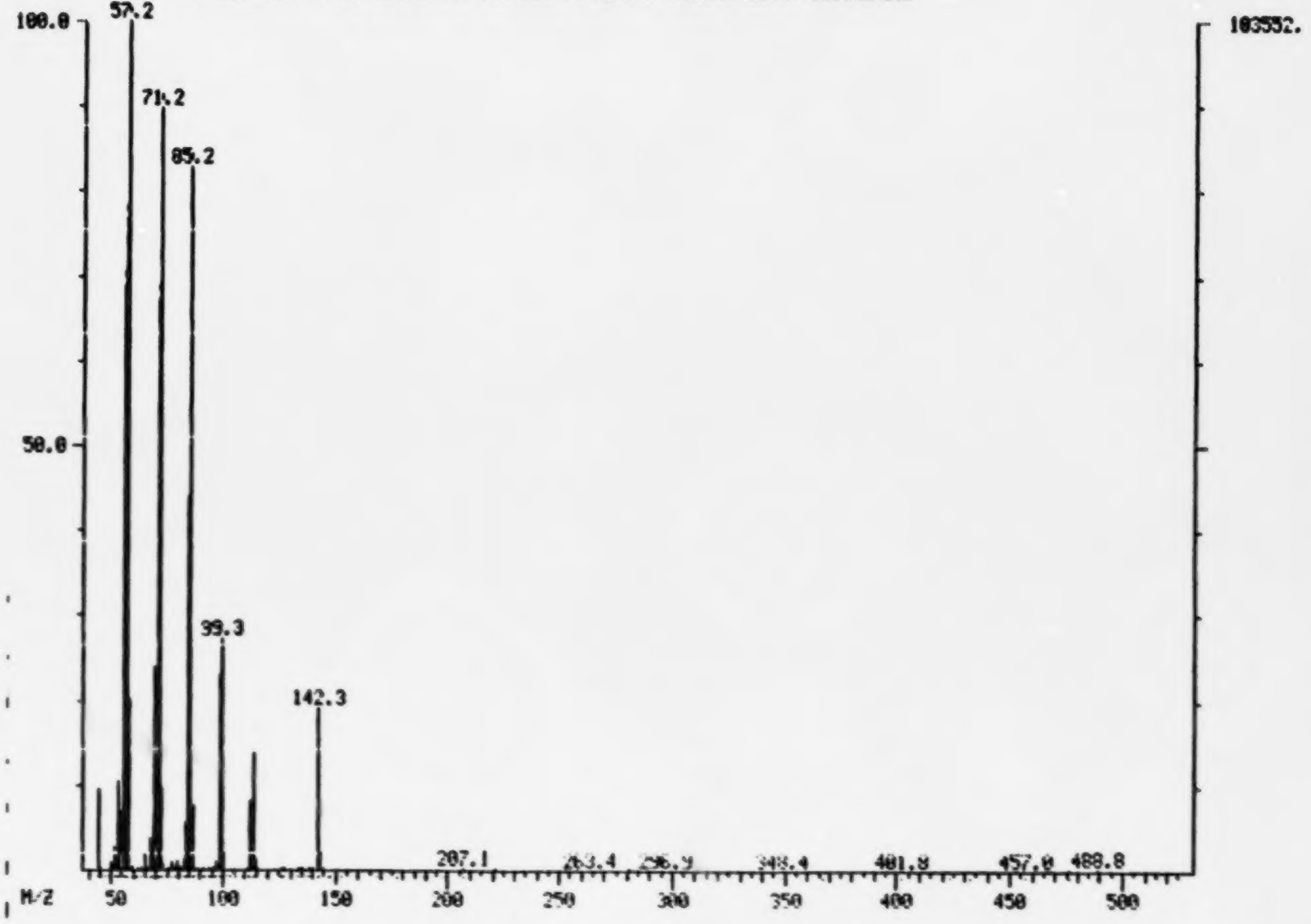
MMS SAMPLE 3
PEAK 1
LIBRARY SEARCH REPORT

LIBRARY SEARCH DATA: 567814 # 502 BASE M/Z: 108
05/07/86 9:54:00 + 5:54 CALI: 567810CAL32 # 4 RIC: 510463.
SAMPLE: 41702-08-03 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI.GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)



MM5 SAMPLE 3
PEAK 1
COMPARISON LIBRARY MASS SPECTRA

```
MASS SPECTRUM                                          DATA: 567814 #660        BASE M/Z:  57
05/07/86  9:54:00 +  7:45                              CALI: 567810CAL32 #4     RIC:    766976.
SAMPLE: 41702-00-03 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
```



MM5 SAMPLE 3
PEAK 2
MASS SPECTRUM

```
Library Search                  Data: 567814 @ 660        Base m/z:     57
05/07/86  9:54:00 +  7:45       Cali: 567810CAL32 @   4   RIC:      754697.
Sample: 41702-08-03 MMS EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI,GC/MS 30MX.25MM DB5 @38779 HE(30) I(300) 60(4)-320@12(20)

39763 spectra in LIBRARYNB searched for maximum PURITY
   357 matched at least 6 of the 16 largest peaks in the unknown

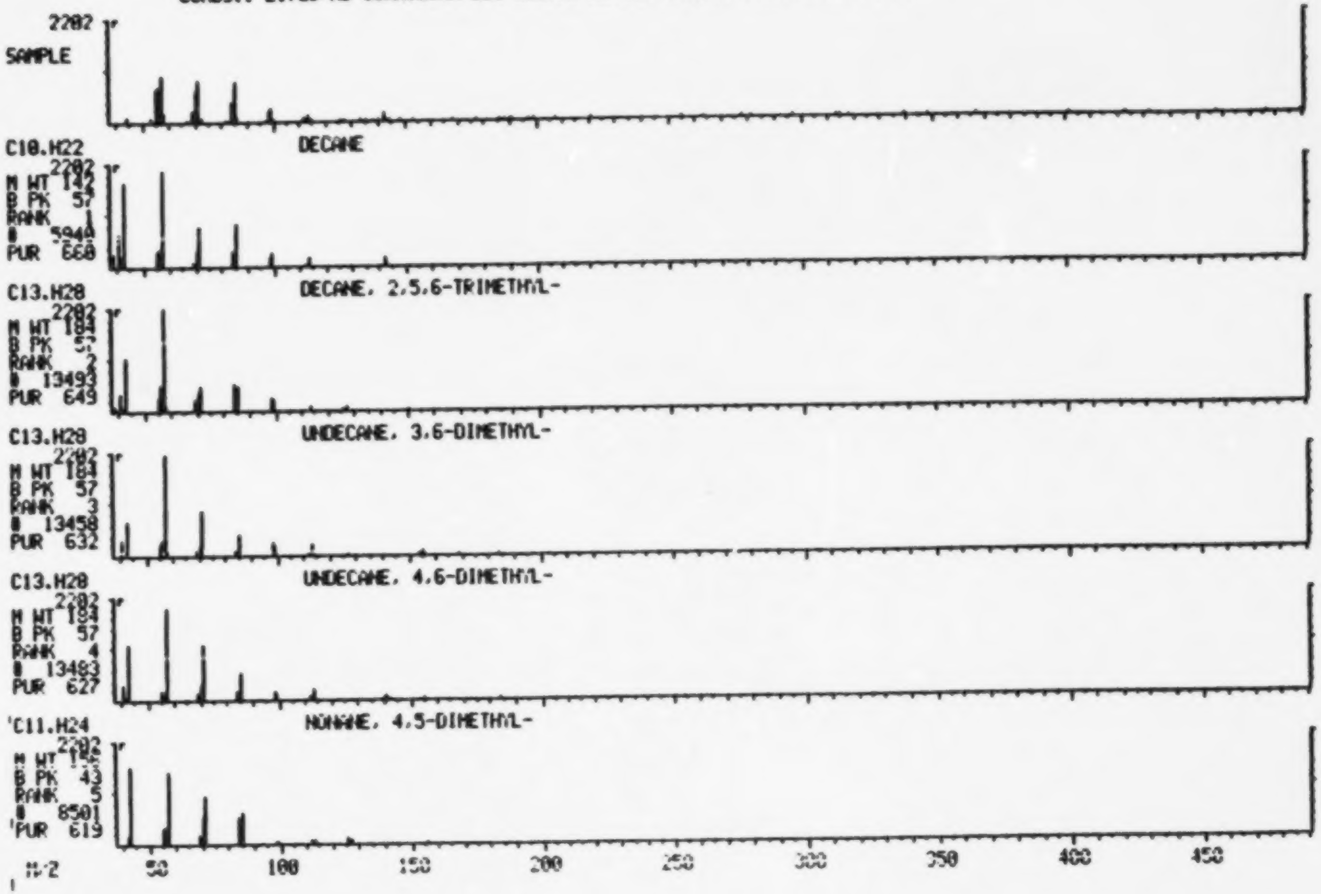
Rank In.       Name
1   5940 DECANE
2  13493 DECANE, 2,5,6-TRIMETHYL-
3  13458 UNDECANE, 3,6-DIMETHYL-
4  13483 UNDECANE, 4,6-DIMETHYL-
5   8501 NONANE, 4,5-DIMETHYL-
6   8506 OCTANE, 2,5,6-TRIMETHYL-
7  13487 UNDECANE, 5,6-DIMETHYL-
8   8502 NONANE, 2,5-DIMETHYL-
9   5959 OCTANE, 4-ETHYL-

Rank   Formula                              M.Wt B.Pk   Purity    Fit    RFit
1      C10.H22                               142   57      660    750     830
2      C13.H28                               184   57      649    783     753
3      C13.H28                               184   57      632    747     737
4      C13.H28                               184   57      627    718     756
5      C11.H24                               156   43      619    752     737
6      C11.H24                               156   57      618    773     690
7      C13.H28                               184   57      617    729     728
8      C11.H24                               156   57      617    729     728
9      C10.H22                               142   57      601    672     762

Rank     Ret.Time     B.P.Int.     US.Par.1     US.Par.2       C.A.S. #
1          ___          ___         0.4580        ___           124-18-5
2          ___          ___         0.3070        ___         62108-23-0
3          ___          ___         0.3240        ___         17301-28-9
4          ___          ___         0.3410        ___         17312-82-2
5          ___          ___         0.4210        ___         17302-23-7
6          ___          ___         0.3180        ___         62016-14-2
7          ___          ___         0.3260        ___         17615-91-7
8          ___          ___         0.3630        ___         17302-27-1
9          ___          ___         0.4050        ___         15869-86-0
```

MMS SAMPLE 3
PEAK 3
LIBRARY SEARCH REPORT

```
LIBRARY SEARCH                                         DATA: 567814 # 660          BASE M/Z:  57
05/07/86  9:54:00 +  7:45                              CALI: 567810CAL32 #   4     RIC:    754687.
SAMPLE: 41702-08-03 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI.GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
```

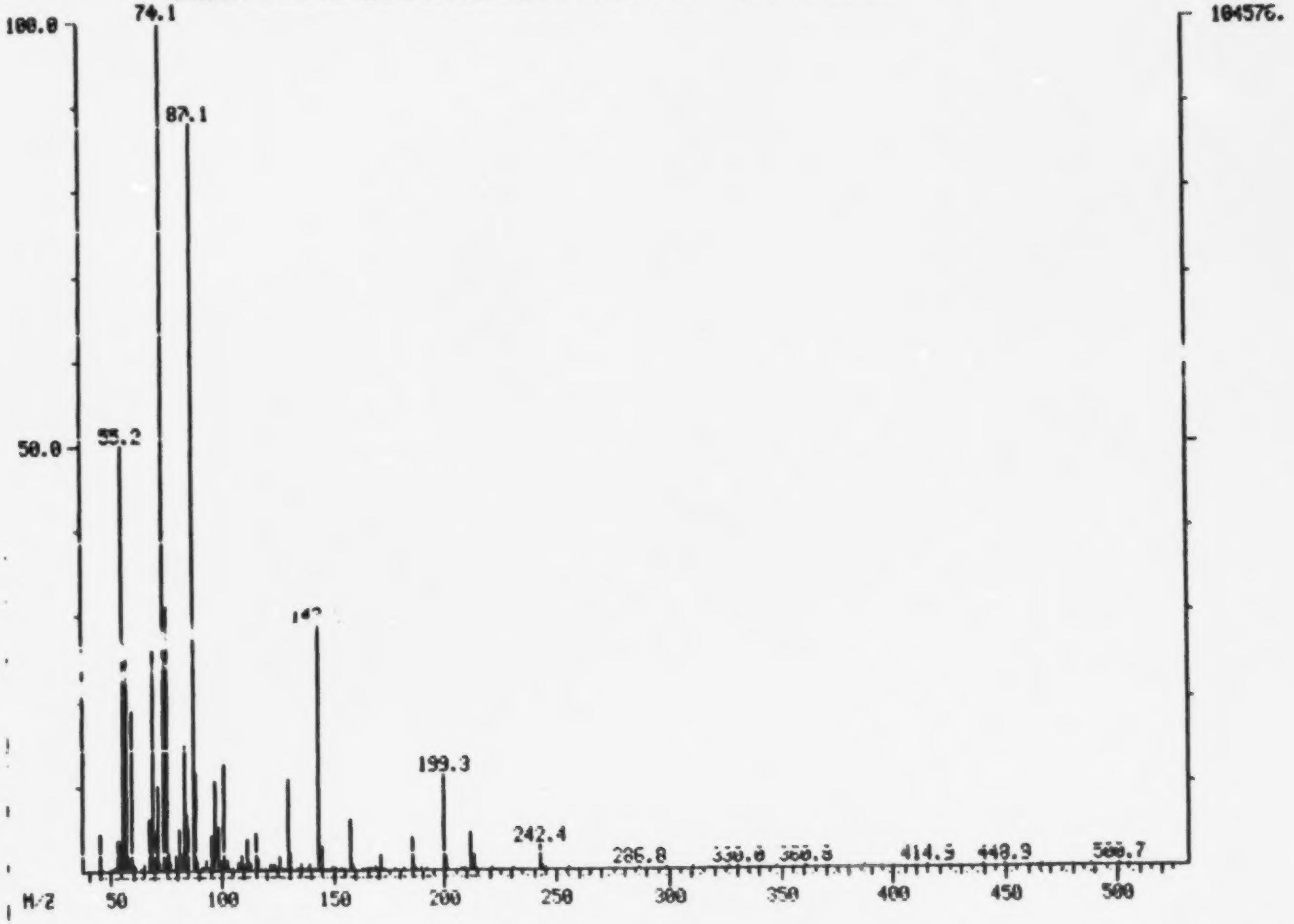


MM5 SAMPLE 3
PEAK 2
COMPARISON LIBRARY MASS SPECTRA

MASS SPECTRUM
05/07/86 9:54:00 + 17:31
SAMPLE: 41702-08-03 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

DATA: 567814 #1492
CALI: 567810CAL32 #4

BASE M/Z: 74
RIC: 613376.



MM5 SAMPLE 3
PEAK 3
MASS SPECTRUM

```
Library Search                 Data: 567814 #1492          Base m/z:    74
05/07/86  9:54:00 + 17:31      Cali: 567810CAL32 #   4     RIC:      578559.
Sample: 41702-08-03 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

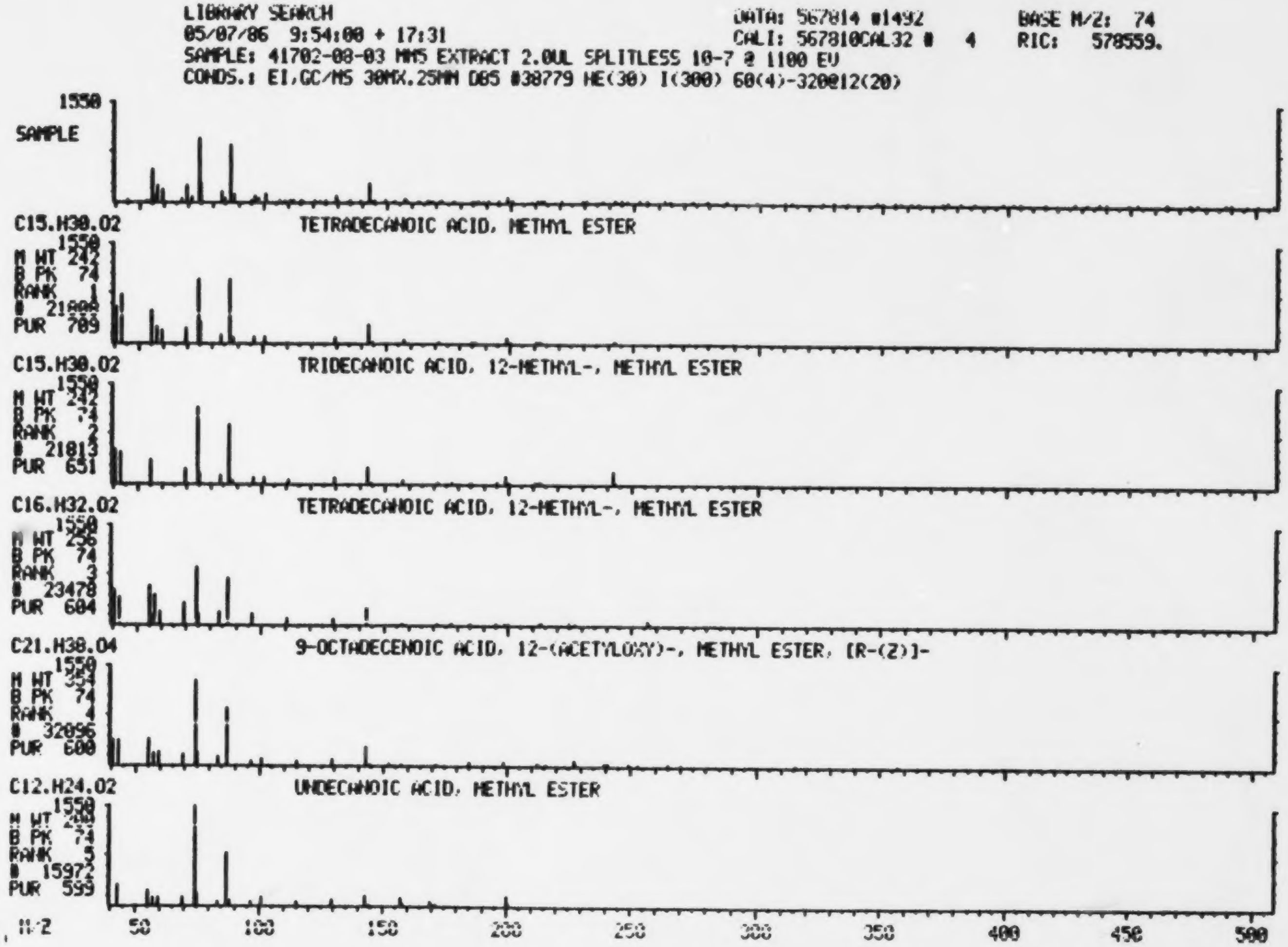
39763 spectra in LIBRARYNB searched for maximum PURITY
  457 matched at least 5 of the 16 largest peaks in the unknown

Rank In.        Name
1 21808 TETRADECANOIC ACID, METHYL ESTER
2 21813 TRIDECANOIC ACID, 12-METHYL-, METHYL ESTER
3 23478 TETRADECANOIC ACID, 12-METHYL-, METHYL ESTER
4 32096 9-OCTADECENOIC ACID, 12-(ACETYLOXY)-, METHYL ESTER, [R-(Z)]-
5 15972 UNDECANOIC ACID, METHYL ESTER
6 36952 TRIACONTANOIC ACID, METHYL ESTER
7 27747 HEPTADECANOIC ACID, 16-METHYL-, METHYL ESTER
8 20119 DODECANOIC ACID, 10-METHYL-, METHYL ESTER
9 18094 UNDECANOIC ACID, 10-METHYL-, METHYL ESTER

Rank  Formula                                M.Wt B.Pk   Purity    Fit   RFit
1     C15.H30.O2                              242   74      709    879    804
2     C15.H30.O2                              242   74      651    855    716
3     C16.H32.O2                              256   74      604    821    702
4     C21.H38.O4                              354   74      600    794    737
5     C12.H24.O2                              200   74      599    836    643
6     C31.H62.O2                              466   74      577    780    710
7     C19.H38.O2                              298   74      574    747    705
8     C14.H28.O2                              228   74      573    767    697
9     C13.H26.O2                              214   74      570    794    639

Rank     Ret Time     B.P.Int     US.Par.1     US.Par.2       C.A.S. #
1          ___          ___        0.6440         ___         124-10-7
2          ___          ___        0.7180         ___        5129-58-8
3          ___          ___        0.6840         ___        5129-66-8
4          ___          ___        0.5950         ___         140-03-4
5          ___          ___        0.6270         ___        1731-86-8
6          ___          ___        0.3330         ___         629-83-4
7          ___          ___        0.6960         ___        5129-61-3
8          ___          ___        0.6300         ___        5129-65-7
9          ___          ___        0.7210         ___        5129-56-6
```

MMS SAMPLE 3
PEAK 3
LIBRARY SEARCH REPORT



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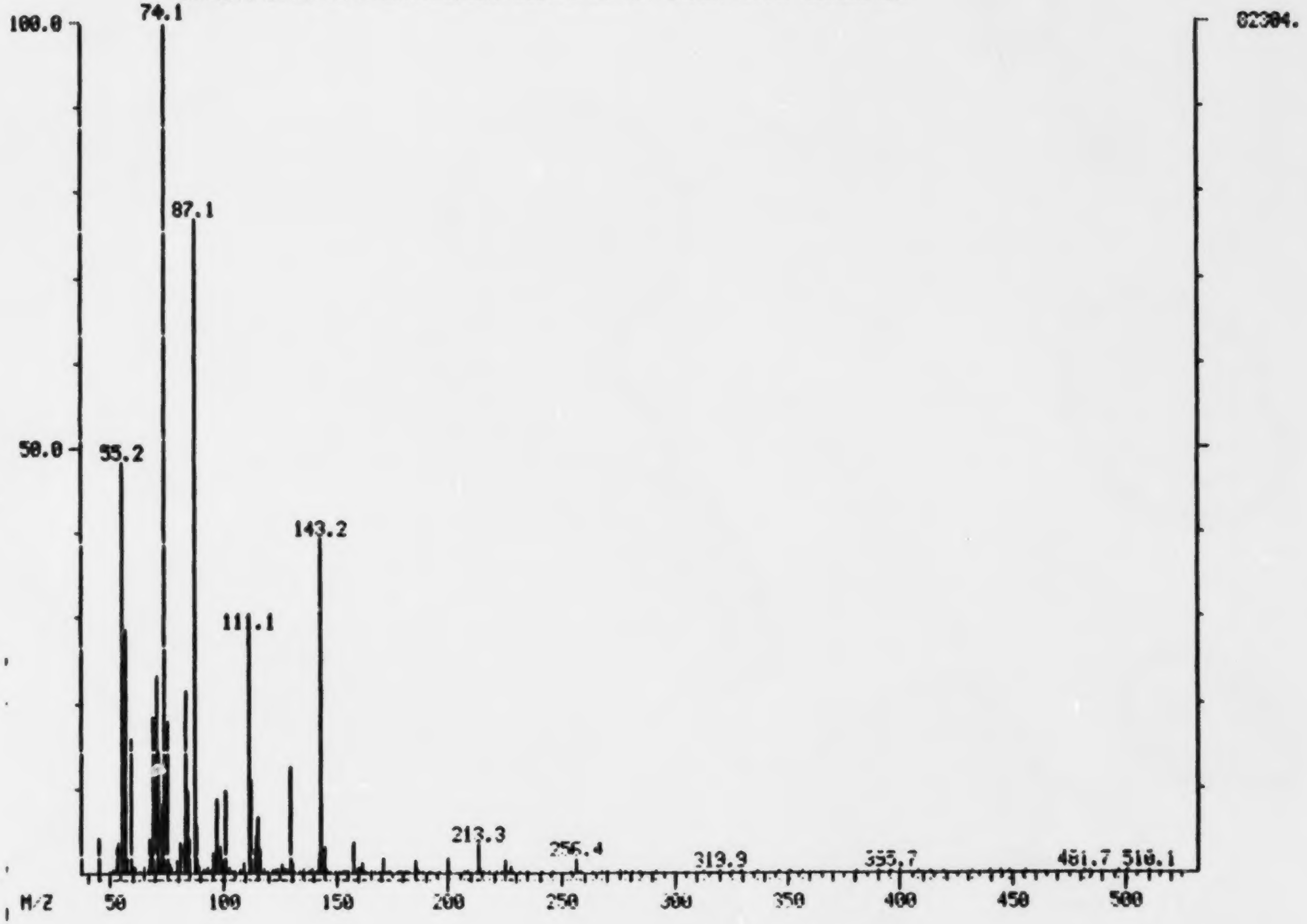
LIBRARY SEARCH                                            DATA: 567814 #1492          BASE M/Z:  74
05/07/86  9:54:00 + 17:31                                 CALI: 567810CAL32 #   4     RIC:    578559.
SAMPLE: 41702-08-03 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
```

MM5 SAMPLE 3
PEAK 3
COMPARISON LIBRARY MASS SPECTRA

MASS SPECTRUM
05/07/86 9:54:00 + 18:32
SAMPLE: 41702-08-03 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

DATA: 567814 #1578
CALI: 567810CAL32 #4

BASE M/Z: 74
RIC: 510976.



MM5 SAMPLE 3
PEAK 4
MASS SPECTRUM

```
Library Search              Data: 567814 #1578         Base m/z:    74
05/07/86  9:54:00 + 18:32   Cali: 567810CAL32 #    4   RIC:     478207.
Sample: 41702-08-03 MMS EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

39763 spectra in LIBRARYNB searched for maximum PURITY
  263 matched at least 6 of the 16 largest peaks in the unknown

Rank In.      Name
1  9039 2-NAPHTHALENOL, 8-AMINO-
2 36952 TRIACONTANOIC ACID, METHYL ESTER
3 27747 HEPTADECANOIC ACID, 16-METHYL-, METHYL ESTER
4 26374 HEXADECANOIC ACID, 14-METHYL-, METHYL ESTER
5 24737 CYCLOPENTANEUNDECANOIC ACID, METHYL ESTER
6 26379 TETRADECANOIC ACID, 5,9,13-TRIMETHYL-, METHYL ESTER
7 13759 OCTANOIC ACID, 4,6-DIMETHYL-, METHYL ESTER, (4S,6S)-(+)-
8 15973 DECANOIC ACID, 8-METHYL-, METHYL ESTER
9 11834 HEXANEDIOIC ACID, DIMETHYL ESTER

Rank  Formula                                M.Wt B.Pk   Purity    Fit    RFit
1     C10.H9.O.N                              159   74      556    850     604
2     C31.H62.O2                              466   74      526    752     666
3     C19.H38 O2                              298   74      512    718     680
4     C18 H36.O2                              284   74      484    706     577
5     C17.H32 O2                              268   74      472    763     574
6     C18.H36.O2                              284   74      463    776     515
7     C11.H22 O2                              186   87      412    794     463
8     C12 H24.O2                              200   74      405    873     459
9     C8 H14 O4                               174   59      374    650     420

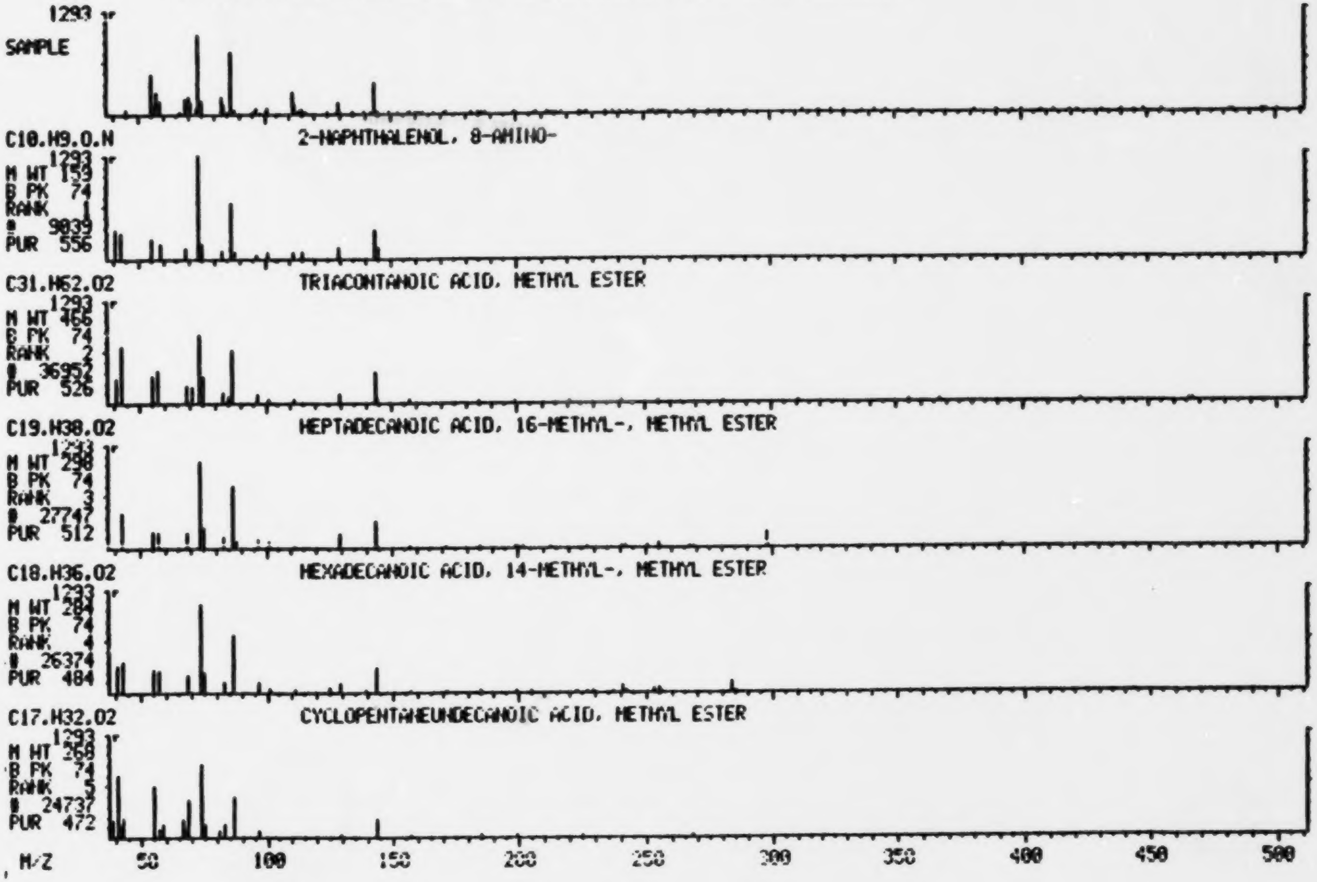
Rank      Ret.Time        B.P.Int.       US.Par.1       US.Par.2        C.A.S. #
1          ____            ____           0.6340          ____           118-46-7
2          ____            ____           0.3330          ____           629-83-4
3          ____            ____           0.6960          ____          5129-61-3
4          ____            ____           0.7160          ____          2490-49-5
5          ____            ____           0.6490          ____         25779-85-5
6          ____            ____           0.5970          ____         56196-55-5
7          ____            ____           0.7160          ____          2553-96-0
8          ____            ____           0.1010          ____          5129-64-6
9          ____            ____           0.7610          ____           627-93-0
```

MMS SAMPLE 3
PEAK 4
LIBRARY SEARCH REPORT

LIBRARY SEARCH
05/07/86 9:54:00 + 18:32
SAMPLE: 41702-08-03 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

DATA: 567814 #1578
CALI: 567810CAL32 # 4

BASE M/Z: 74
RIC: 478207.



MM5 SAMPLE 3
PEAK 4
COMPARISON LIBRARY MASS SPECTRA

```
MASS SPECTRUM                                      DATA: 567814 #1642        BASE M/Z:  55
05/07/86  9:54:00 + 19:17                          CALI: 567810CAL32 #4      RIC:   730112.
SAMPLE: 41702-08-03 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
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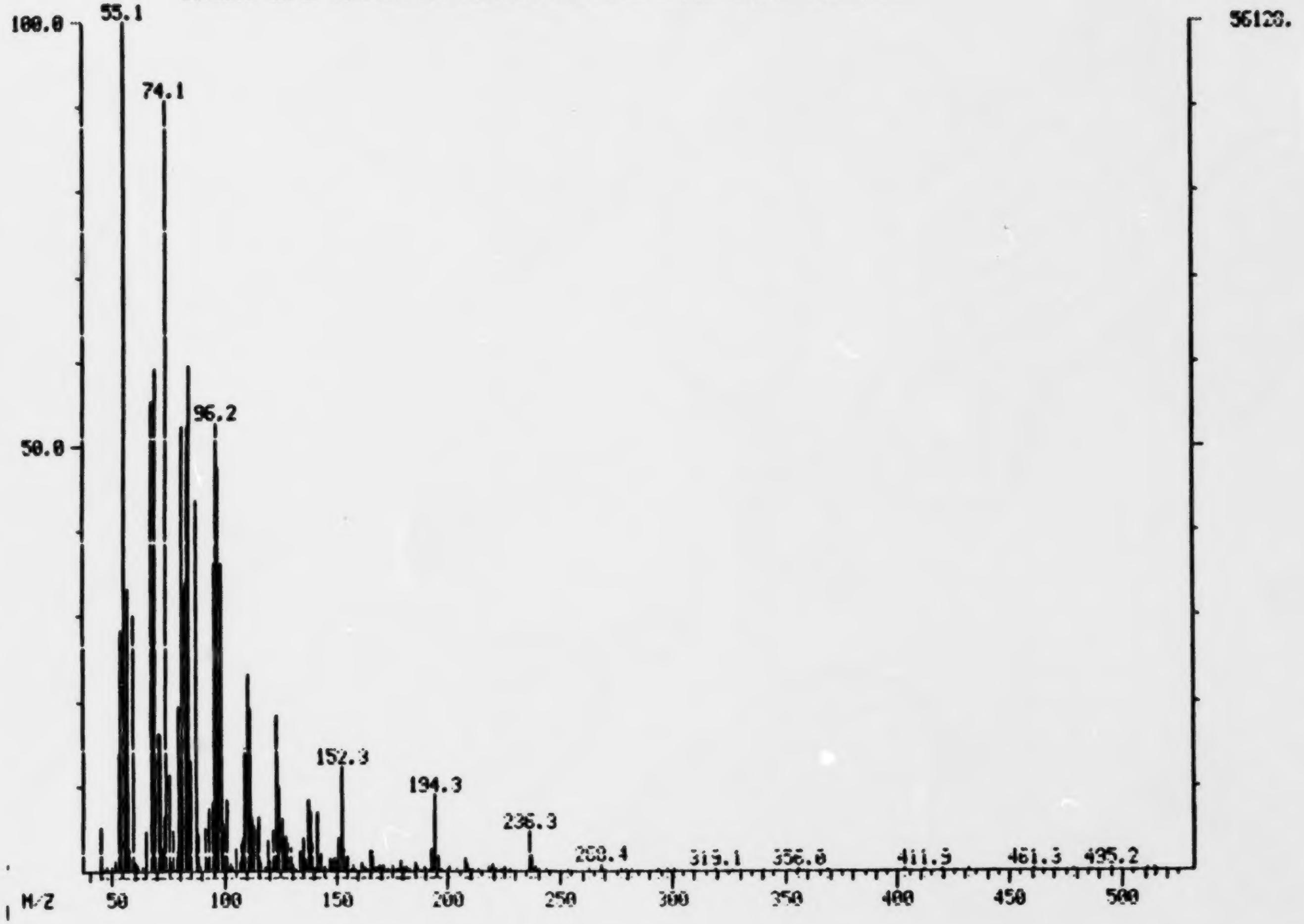

MMS SAMPLE 3
PEAK 5
MASS SPECTRUM

```
Library Search                Data: 567814 #1642        Base m/z:    55
05/07/86  9:54:00 + 19:17     Cali: 567810CAL32 #   4   RIC:     623615.
Sample: 41702-08-03 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

39763 spectra in LIBRARYNB searched for maximum PURITY
  121 matched at least 7 of the 16 largest peaks in the unknown

Rank In.        Name
1 16233 10-UNDECENOYL CHLORIDE
2 28996 ETHANOL, 2-(9-OCTADECENYLOXY)-, (Z)-
3 25811 9-EICOSYNE
4 14980 1,13-TETRADECADIENE
5 27358 13,16-OCTADECADIENOIC ACID, METHYL ESTER
6 28999 ETHANOL, 2-(9-OCTADECENYLOXY)-, (E)-
7 25810 3-EICOSYNE
8 21535 OXACYCLOTETRADECANE-2,11-DIONE, 13-METHYL-
9 25804 1-EICOSYNE

Rank  Formula                                 M.Wt B.Pk   Purity    Fit   RFit
1     C11.H19.O.CL                             202   55      592    951    597
2     C20.H40.O2                               312   55      517    863    578
3     C20.H38                                  278   81      510    854    560
4     C14.H26                                  194   55      509    898    542
5     C19.H34.O2                               294   55      499    852    556
6     C20.H40.O2                               312   55      498    860    556
7     C20.H38                                  278   67      487    815    519
8     C14.H24.O3                               240   55      479    808    567
9     C20.H38                                  278   43      477    809    555

Rank      Ret.Time     B.P.Int.      US.Par.1     US.Par.2       C.A.S. #
1          ____         ____          0.3840        ____        38460-95-6
2          ____         ____          0.6760        ____         5353-25-3
3          ____         ____          0.7130        ____        71899-38-2
4          ____         ____          0.6100        ____        21964-49-8
5          ____         ____          0.7190        ____        56846-99-2
6          ____         ____          0.7200        ____        17367-07-6
7          ____         ____          0.6960        ____        61836-66-6
8          ____         ____          0.7980        ____        74685-36-2
9          ____         ____          0.3920        ____          765-27-5
```

MM5 SAMPLE 3
PEAK 5
LIBRARY SEARCH REPORT

```
LIBRARY SEARCH                                        DATA: 567814 #1642        BASE M/Z:  55
05/07/86  9:54:00 + 19:17                             CALI: 567810CAL32 #   4   RIC:    623615.
SAMPLE: 41702-08-03 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
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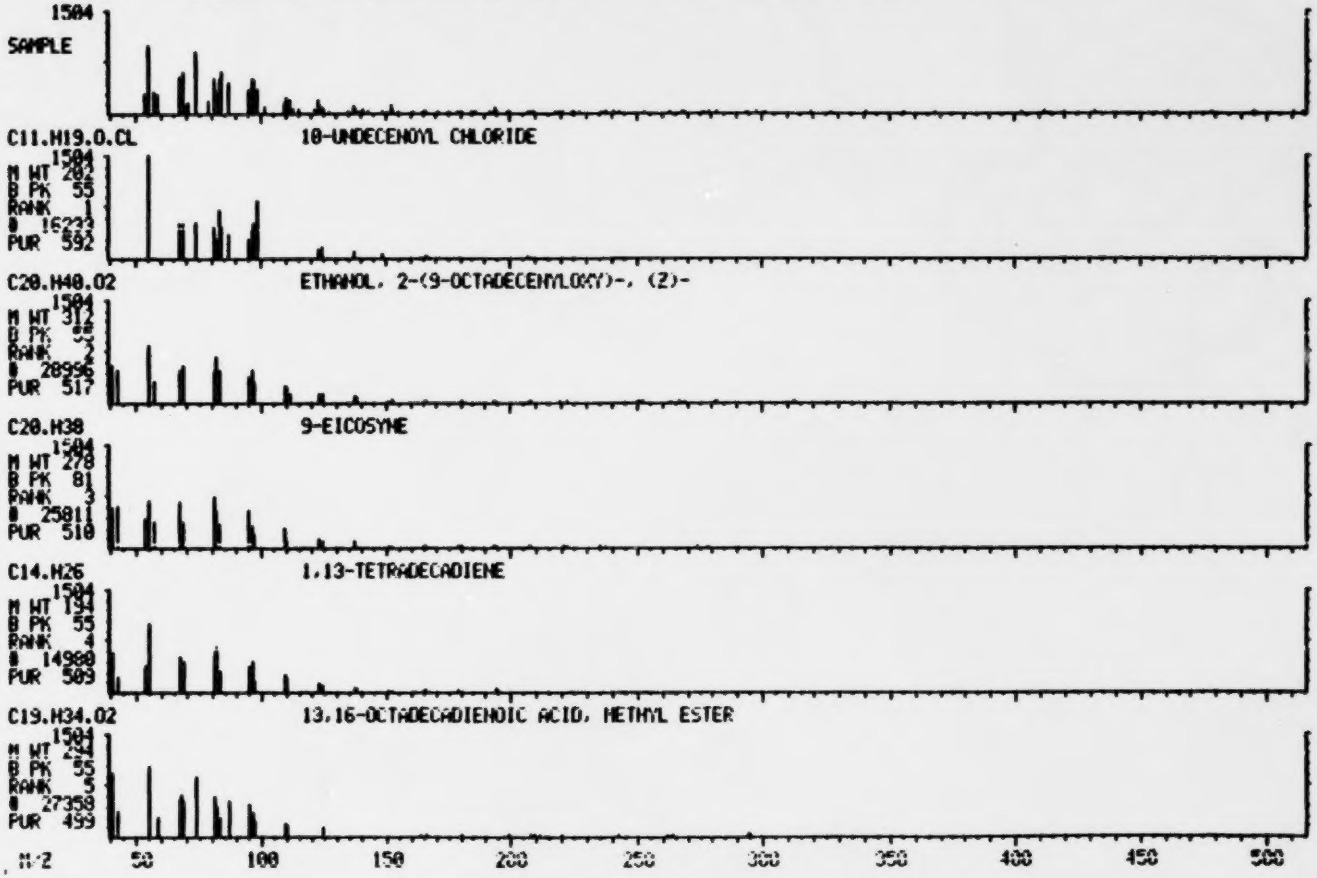
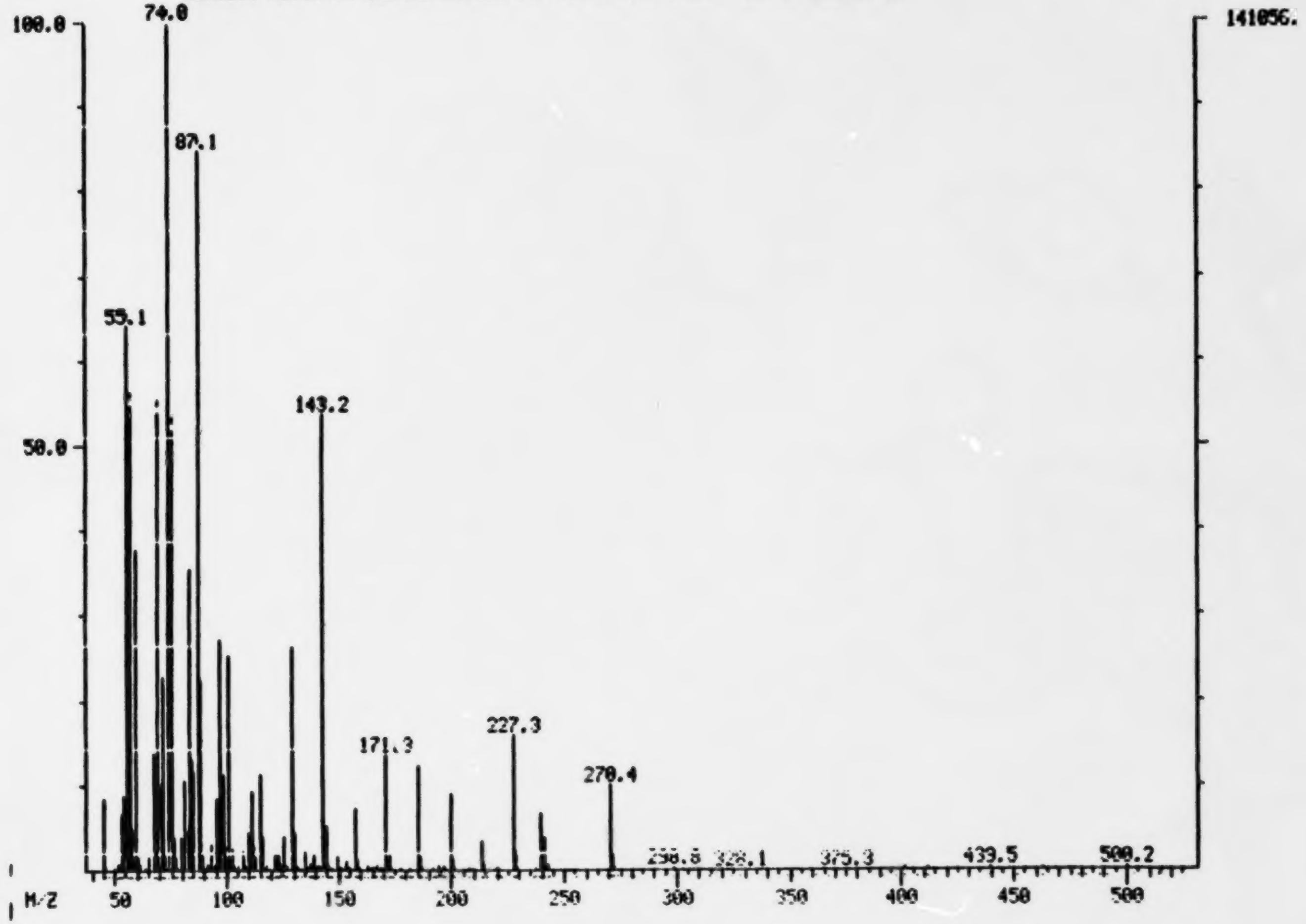


MM5 SAMPLE 3
PEAK 5
COMPARISON LIBRARY MASS SPECTRA

MASS SPECTRUM
05/07/86 9:54:00 + 19:30
SAMPLE: 41702-08-03 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

DATA: 567814 #1661
CALI: 567810CAL32 #4

BASE M/Z: 74
RIC: 1394680.



MM5 SAMPLE 3
PEAK 6
MASS SPECTRUM

```
Library Search                Data: 567814 #1661          Base m/z:    74
05/07/86  9:54:00 + 19:30     Cali: 567810CAL32 #    4    RIC:    1300470.
Sample: 41702-08-03 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

39763 spectra in LIBRARYNB searched for maximum PURITY
  402 matched at least 5 of the 16 largest peaks in the unknown

Rank In.      Name
1 32096 9-OCTADECENOIC ACID, 12-(ACETYLOXY)-, METHYL ESTER, [R-(Z)]-
2 24977 PENTADECANOIC ACID, 14-METHYL-, METHYL ESTER
3 24978 PENTADECANOIC ACID, 13-METHYL-, METHYL ESTER
4 24975 HEXADECANOIC ACID, METHYL ESTER
5 23478 TETRADECANOIC ACID, 12-METHYL-, METHYL ESTER
6 20119 DODECANOIC ACID, 10-METHYL-, METHYL ESTER
7 21808 TETRADECANOIC ACID, METHYL ESTER
8 18094 UNDECANOIC ACID, 10-METHYL-, METHYL ESTER
9 37817 OCTADECANOIC ACID, 3-HYDROXY-2-TETRADECYL-, METHYL ESTER

Rank  Formula                                M.Wt B.Pk   Purity    Fit    RFit
1     C21.H38.O4                              354   74      662    861     734
2     C17.H34.O2                              270   74      647    836     702
3     C17.H34.O2                              270   74      636    829     687
4     C17.H34.O2                              270   74      621    832     705
5     C16.H32.O2                              256   74      536    785     634
6     C14.H28.O2                              228   74      533    795     596
7     C15.H30.O2                              242   74      530    780     626
8     C13.H26.O2                              214   74      527    799     583
9     C33.H66.O3                              510   43      518    762     579

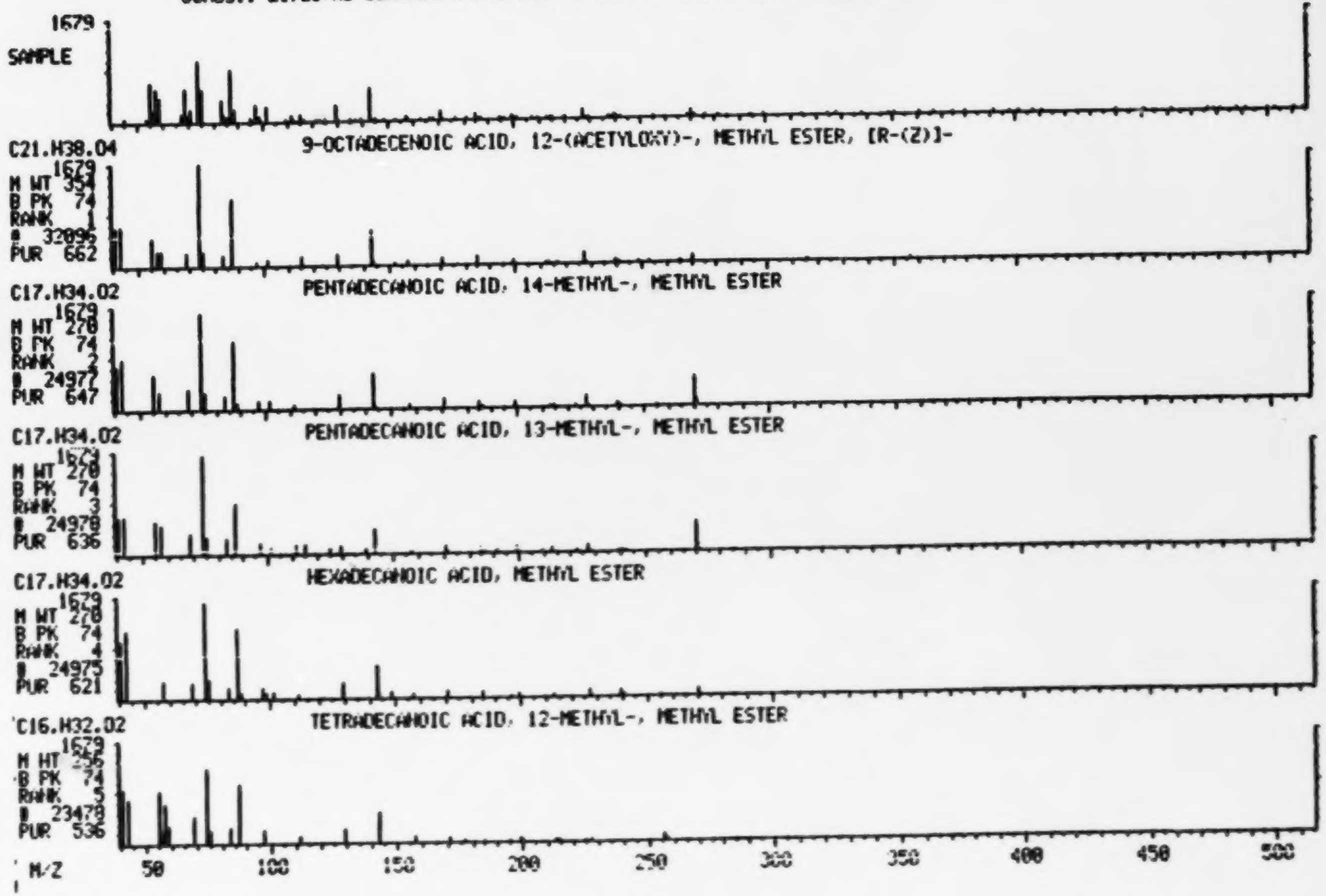
Rank      Ret.Time      B.P.Int.      US.Par.1      US.Par.2       C.A.S. #
1                                       0.5950                     140-03-4
2           ____          ____          0.7090        ____        5129-60-2
3           ____          ____          0.6430        ____        5487-50-3
4           ____          ____          0.7140        ____         112-39-0
5           ____          ____          0.6840        ____        5129-66-8
6           ____          ____          0.6300        ____        5129-65-7
7           ____          ____          0.6440        ____         124-10-7
8           ____          ____          0.7210        ____        5129-56-6
9           ____          ____          0.6910        ____       17369-87-8
            ____          ____                        
```

MM5 SAMPLE 3
PEAK 6
LIBRARY SEARCH REPORT

LIBRARY SEARCH
05/07/86 9:54:00 + 19:30
SAMPLE: 41702-08-03 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

DATA: 567814 #1661
CALI: 567810CAL32 # 4

BASE M/Z: 74
RIC: 1300470.

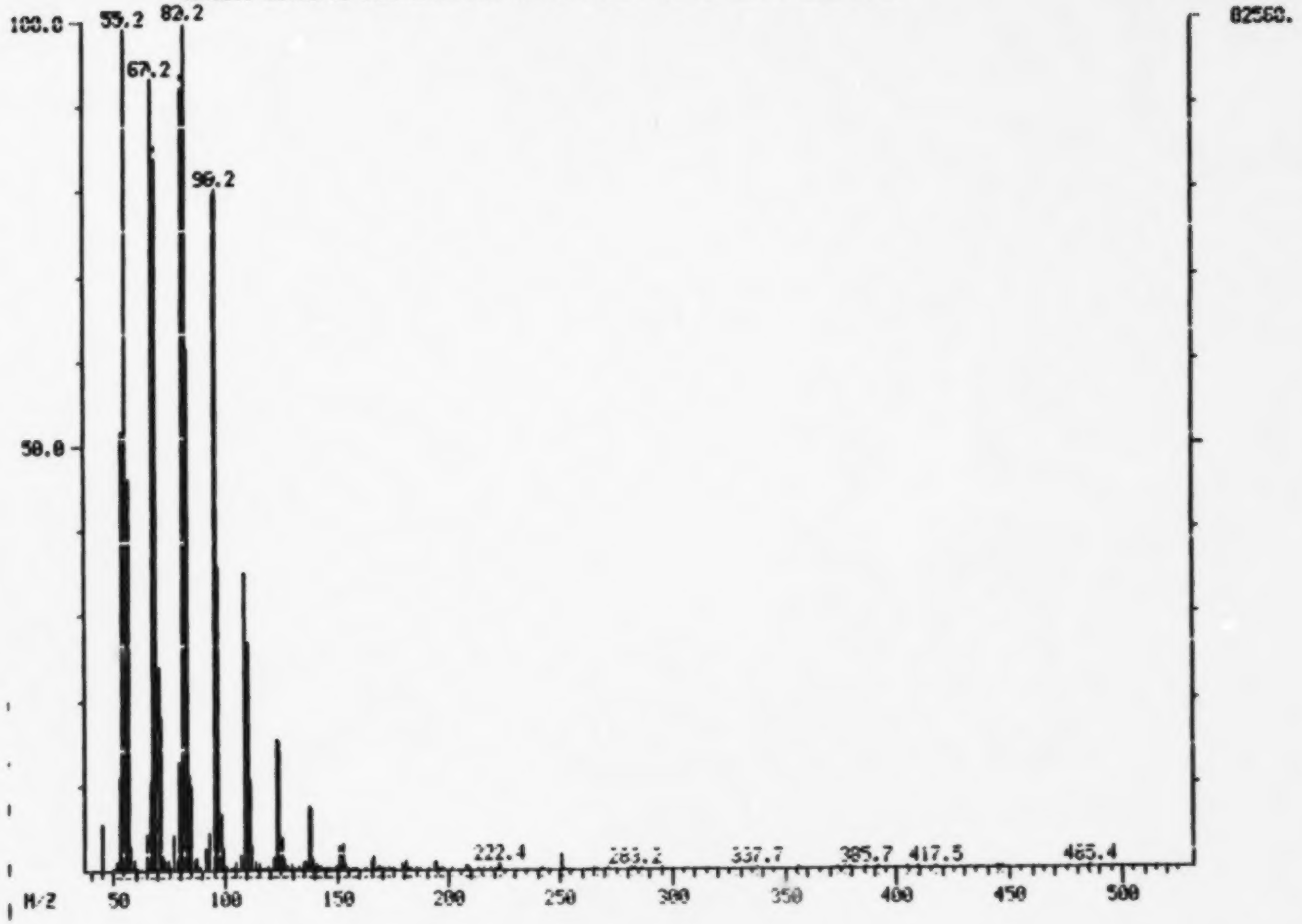


MM5 SAMPLE 3
PEAK 6
COMPARISON LIBRARY MASS SPECTRA

MASS SPECTRUM
05/07/86 9:54:00 + 20:43
SAMPLE: 41702-08-03 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI.GC/MS 30MX.25MM DB5 038779 HE(30) I(300) 60(4)-320@12(20)

DATA: 567814 #1764
CALI: 567810CAL32 #4

BASE M/Z: 82
RIC: 1017850.



MM5 SAMPLE 3
PEAK 7
MASS SPECTRUM

```
Library Search                    Data: 367814 01764          Base m/z:    82
05/07/86  9:34:00 + 20:43         Cali: 367810CAL32 #    4    RIC:      970751.
Sample: 41702-08-03 MMS EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

39763 spectra in LIBRARYNB searched for maximum PURITY
  266 matched at least 7 of the 16 largest peaks in the unknown

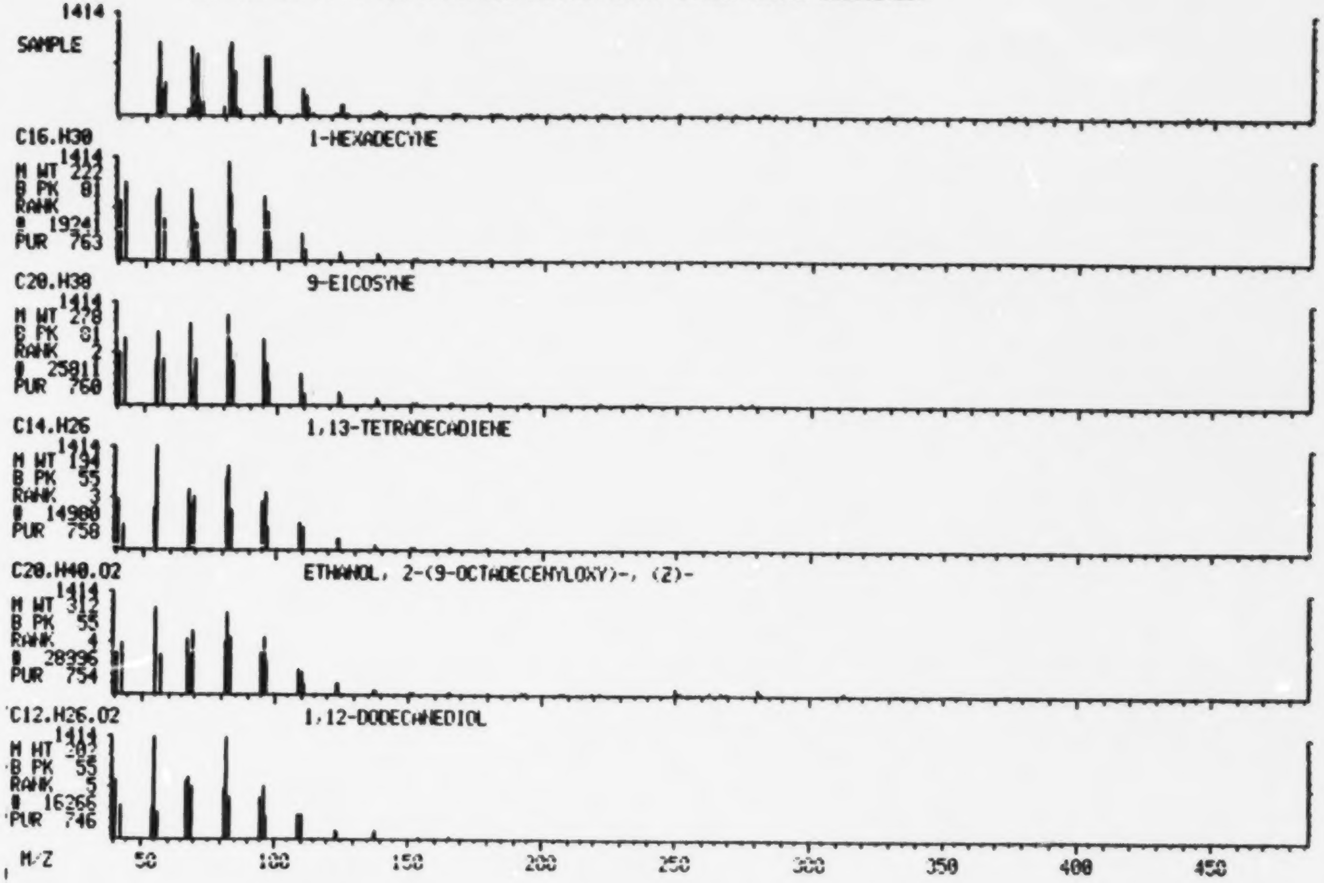
Rank In.       Name
1 19241 1-HEXADECYNE
2 25811 9-EICOSYNE
3 14980 1,13-TETRADECADIENE
4 28996 ETHANOL, 2-(9-OCTADECENYLOXY)-, (Z)-
5 16266 1,12-DODECANEDIOL
6 24764 9-OCTADECEN-1-OL, (Z)-
7 28999 ETHANOL, 2-(9-OCTADECENYLOXY)-, (E)-
8 10310 1,11-DODECADIENE
9 12765 1,12-TRIDECADIENE

Rank   Formula                                    M.Wt  B.Pk    Purity     Fit    RFit
1      C16.H30                                     222   81       763      893     837
2      C20.H38                                     278   81       760      906     826
3      C14.H26                                     194   55       758      942     800
4      C20.H40.O2                                  312   55       754      911     812
5      C12.H26.O2                                  202   55       746      921     795
6      C18.H36.O                                   268   55       745      911     798
7      C20.H40.O2                                  312   82       743      909     779
8      C12.H22                                     166   55       736      925     785
9      C13.H24                                     180   55       734      924     785

Rank      Ret.Time      B.P.Int       US.Par.1      US.Par.2       C.A.S. #
1           ____          ____         0.4570         ____           629-74-3
2           ____          ____         0.7130         ____         71899-38-2
3           ____          ____         0.6100         ____         21964-49-8
4           ____          ____         0.6760         ____          5353-25-3
5           ____          ____         0.6910         ____          5675-51-4
6           ____          ____         0.7120         ____           143-28-2
7           ____          ____         0.7200         ____         17367-07-6
8           ____          ____         0.5970         ____          5876-87-9
9           ____          ____         0.5330         ____         21964-48-7
```

MMS SAMPLE 3
PEAK 7
LIBRARY SEARCH REPORT

```
LIBRARY SEARCH                                          DATA: 567814 #1764          BASE M/Z:  82
05/07/86  9:54:00 + 20:43                               CALI: 567810CAL32 #   4     RIC:    970751.
SAMPLE: 41702-08-03 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
```

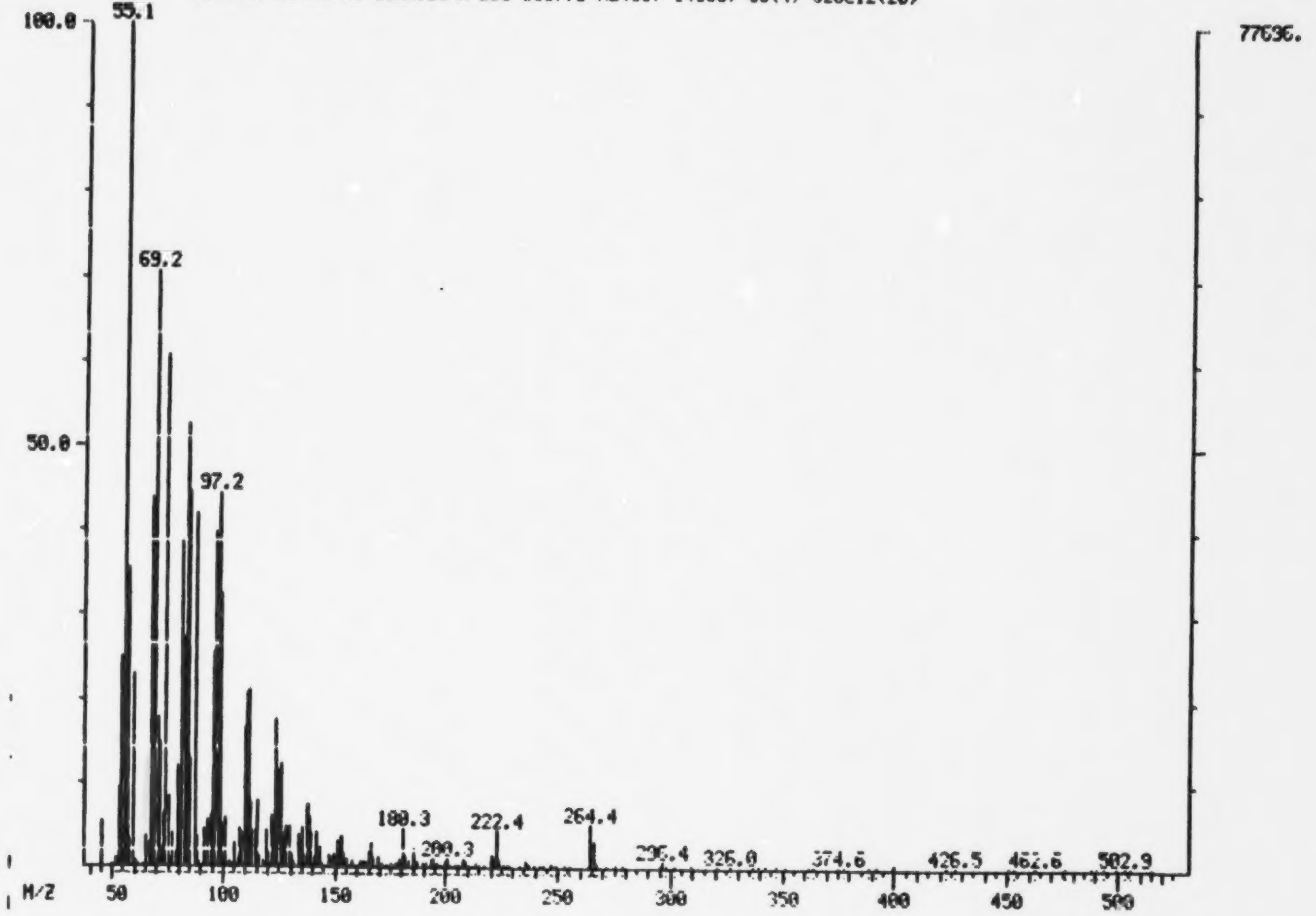


MM5 SAMPLE 3
PEAK 7
COMPARISON LIBRARY MASS SPECTRA

MASS SPECTRUM
05/07/86 9:54:00 + 21:03
SAMPLE: 41702-08-03 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

DATA: 567814 #1793
CALI: 567810CAL32 #4

BASE M/Z: 55
RIC: 921600.



MM5 SAMPLE 3
PEAK 8
MASS SPECTRUM

```
Library Search              Data: 567814 #1793        Base m/z:    55
05/07/86  9:54:00 + 21:03   Cali: 567810CAL32 #   4   RIC:     777215.
Sample: 41702-08-03 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

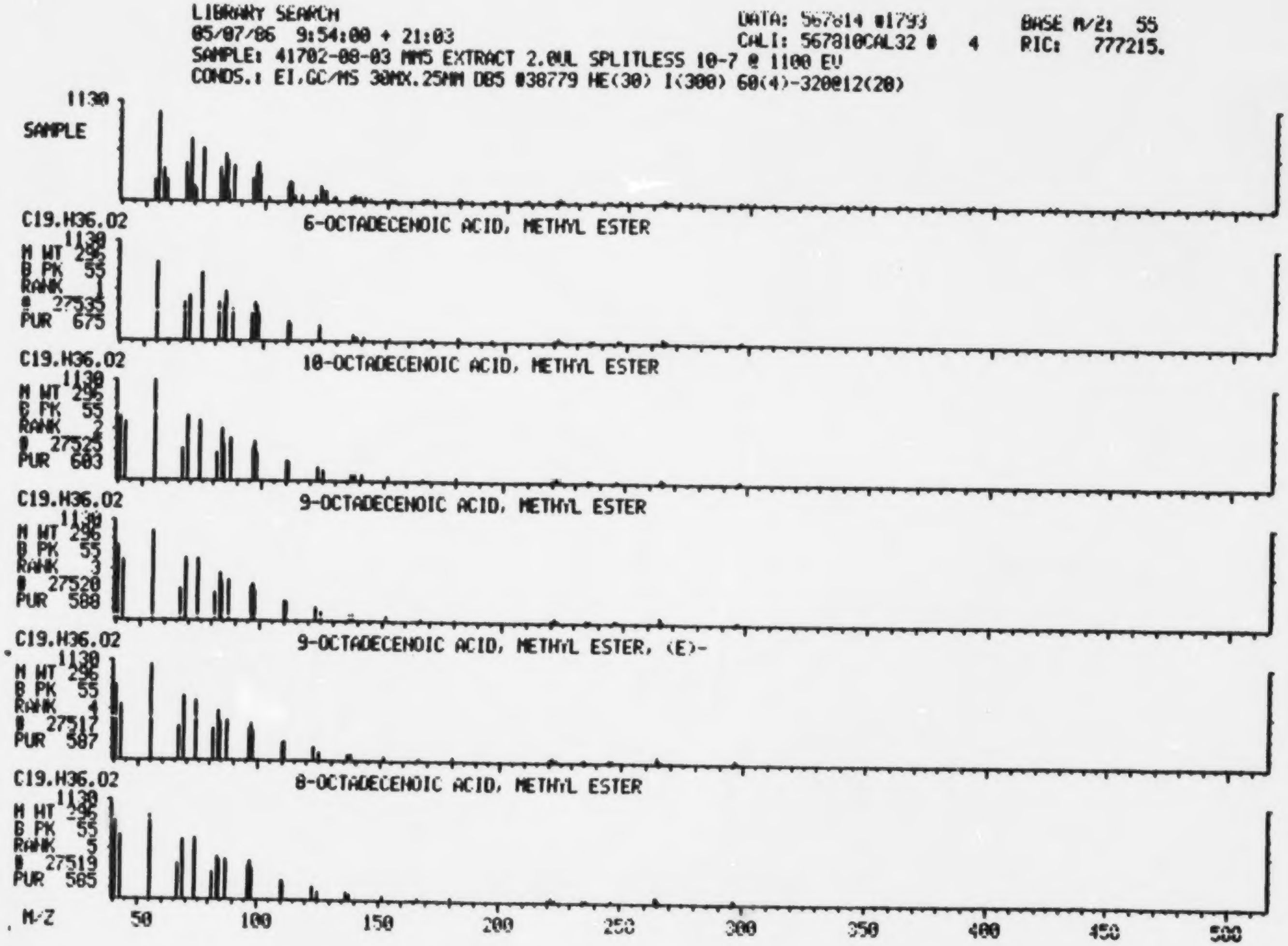
39763 spectra in LIBRARYNB searched for maximum PURITY
  443 matched at least 6 of the 16 largest peaks in the unknown

Rank In.      Name
1 27535 6-OCTADECENOIC ACID, METHYL ESTER
2 27525 10-OCTADECENOIC ACID, METHYL ESTER
3 27520 9-OCTADECENOIC ACID, METHYL ESTER
4 27517 9-OCTADECENOIC ACID, METHYL ESTER, (E)-
5 27519 8-OCTADECENOIC ACID, METHYL ESTER
6 27539 13-OCTADECENOIC ACID, METHYL ESTER
7 27518 11-OCTADECENOIC ACID, METHYL ESTER, (Z)-
8 16233 10-UNDECENOYL CHLORIDE
9 27538 12-OCTADECENOIC ACID, METHYL ESTER

Rank   Formula                          M.Wt  B.Pk   Purity    Fit   RFit
1      C19.H36.O2                        296   55      675     992    675
2      C19.H36.O2                        296   55      603     909    661
3      C19.H36.O2                        296   55      588     895    651
4      C19.H36.O2                        296   55      587     896    650
5      C19.H36.O2                        296   55      585     890    651
6      C19.H36.O2                        296   55      572     923    615
7      C19.H36.O2                        296   55      570     903    625
8      C11.H19.O.CL                      202   55      570     966    570
9      C19.H36.O2                        296   55      566     906    616

Rank     Ret.Time     B.P.Int.     US Par.1    US.Par.2      C.A.S. #
1          ___          ___         0.7190       ___        52355-31-4
2          ___          ___         0.6890       ___        13481-95-3
3          ___          ___         0.7260       ___         2462-84-2
4          ___          ___         0.7260       ___         1937-62-8
5          ___          ___         0.7200       ___         2345-29-1
6          ___          ___         0.6580       ___        56554-47-3
7          ___          ___         0.7200       ___         1937-63-9
8          ___          ___         0.8840       ___        38460-95-6
9          ___          ___         0.7180       ___        56554-46-2
```

MM5 SAMPLE 3
PEAK 8
LIBRARY SEARCH REPORT



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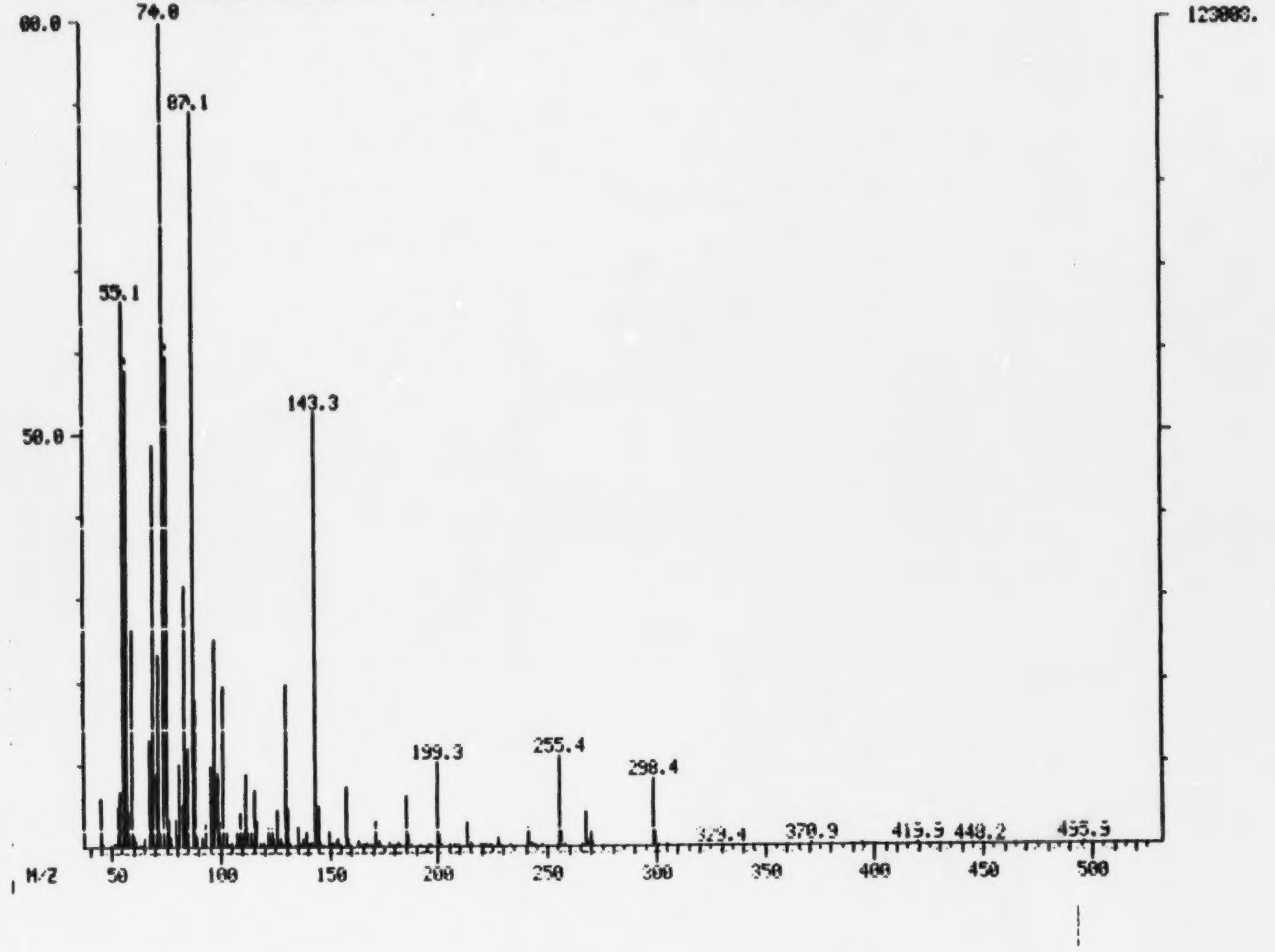
LIBRARY SEARCH                                               DATA: 567814 #1793          BASE M/Z:  55
05/07/86  9:54:00 + 21:03                                    CALI: 567810CAL32 #   4     RIC:    777215.
SAMPLE: 41702-08-03 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
```

MM5 SAMPLE 3
PEAK 8
COMPARISON LIBRARY MASS SPECTRA

```
MASS SPECTRUM                                          DATA: 567814 #1811          BASE M/Z:  74
05/07/86  9:54:00 + 21:16                              CALI: 567810CAL32 #4        RIC:  1138680.
SAMPLE: 41702-08-03 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
```



MM5 SAMPLE 3
PEAK 9
MASS SPECTRUM

```
Library Search               Data: 567814 01811          Base m/z:    74
05/07/86  9:54:00 + 21:16    Cali: 567810CAL32 #   4   RIC:    1043450.
Sample: 41702-08-03 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI.GC/MS 30MX.25MM DB5 038779 HE(30) I(300) 60(4)-320@12(20)

39763 spectra in LIBRARYNB searched for maximum PURITY
  127 matched at least 6 of the 16 largest peaks in the unknown

Rank In.       Name
1 27747 HEPTADECANOIC ACID, 16-METHYL-, METHYL ESTER
2 27752 HEPTADECANOIC ACID, 15-METHYL-, METHYL ESTER
3 27745 HEPTADECANOIC ACID, 14-METHYL-, METHYL ESTER
4 27759 HEPTADECANOIC ACID, 14-METHYL-, METHYL ESTER, (.+-.)-
5 27746 HEPTADECANOIC ACID, 10-METHYL-, METHYL ESTER
6 27754 HEPTADECANOIC ACID, 9-METHYL-, METHYL ESTER
7 36952 TRIACONTANOIC ACID, METHYL ESTER
8 21308 TETRADECANOIC ACID, METHYL ESTER
9 27741 OCTADECANOIC ACID, METHYL ESTER

Rank  Formula                                M.Wt B.Pk   Purity    Fit   RFit
1     C19.H38.02                              298   74      705    911    747
2     C19.H38.02                              298   74      674    886    696
3     C19.H38.02                              298   74      636    832    679
4     C19.H38.02                              298   74      635    831    679
5     C19.H38.02                              298   74      626    817    675
6     C19.H38 02                              298   74      595    784    704
7     C31.H62.02                              466   74      549    777    689
8     C15.H30 02                              242   74      541    795    640
9     C19.H38.02                              298   74      525    755    620

Rank     Ret.Time     B.P.Int.     US.Par.1     US.Par.2      C.A.S. #
1         ____         ____        0.6960        ____         5129-61-3
2         ____         ____        0.7130        ____        54833-55-5
3         ____         ____        0.7110        ____         2490-23-5
4         ____         ____        0.7070        ____        57274-45-0
5         ____         ____        0.6670        ____         2490-25-7
6         ____         ____        0.7140        ____        54934-57-5
7         ____         ____        0.3330        ____          629-83-4
8         ____         ____        0 6440        ____          124-10-7
9         ____         ____        0.7170        ____          112-61-8
```

MM5 SAMPLE 3
PEAK 9
LIBRARY SEARCH REPORT

```
LIBRARY SEARCH                                           DATA: 567814 #1811          BASE M/Z:  74
05/07/86  9:54:00 + 21:16                                CALI: 567810CAL32 #   4     RIC:   1043450.
SAMPLE: 41702-08-03 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EU
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
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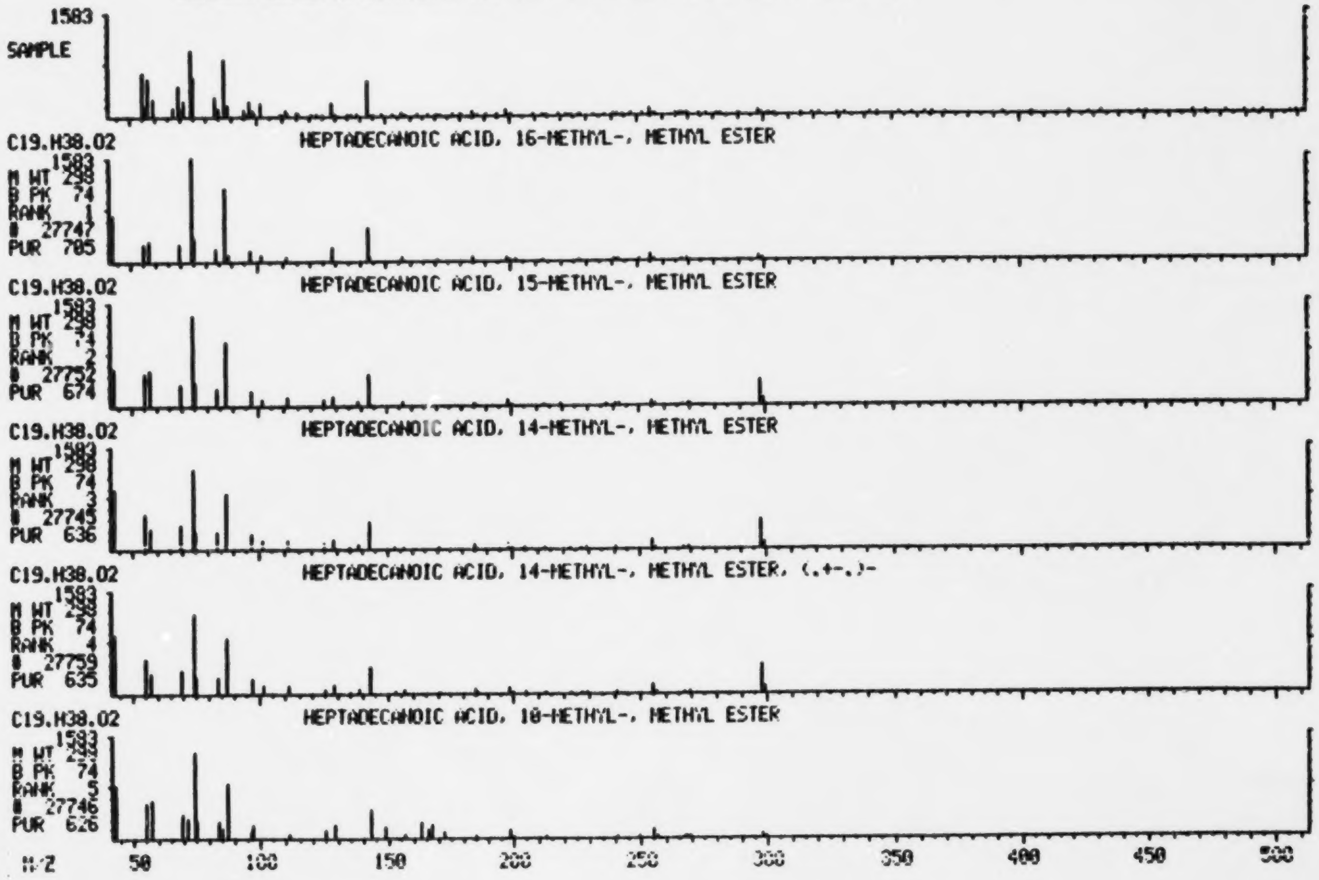
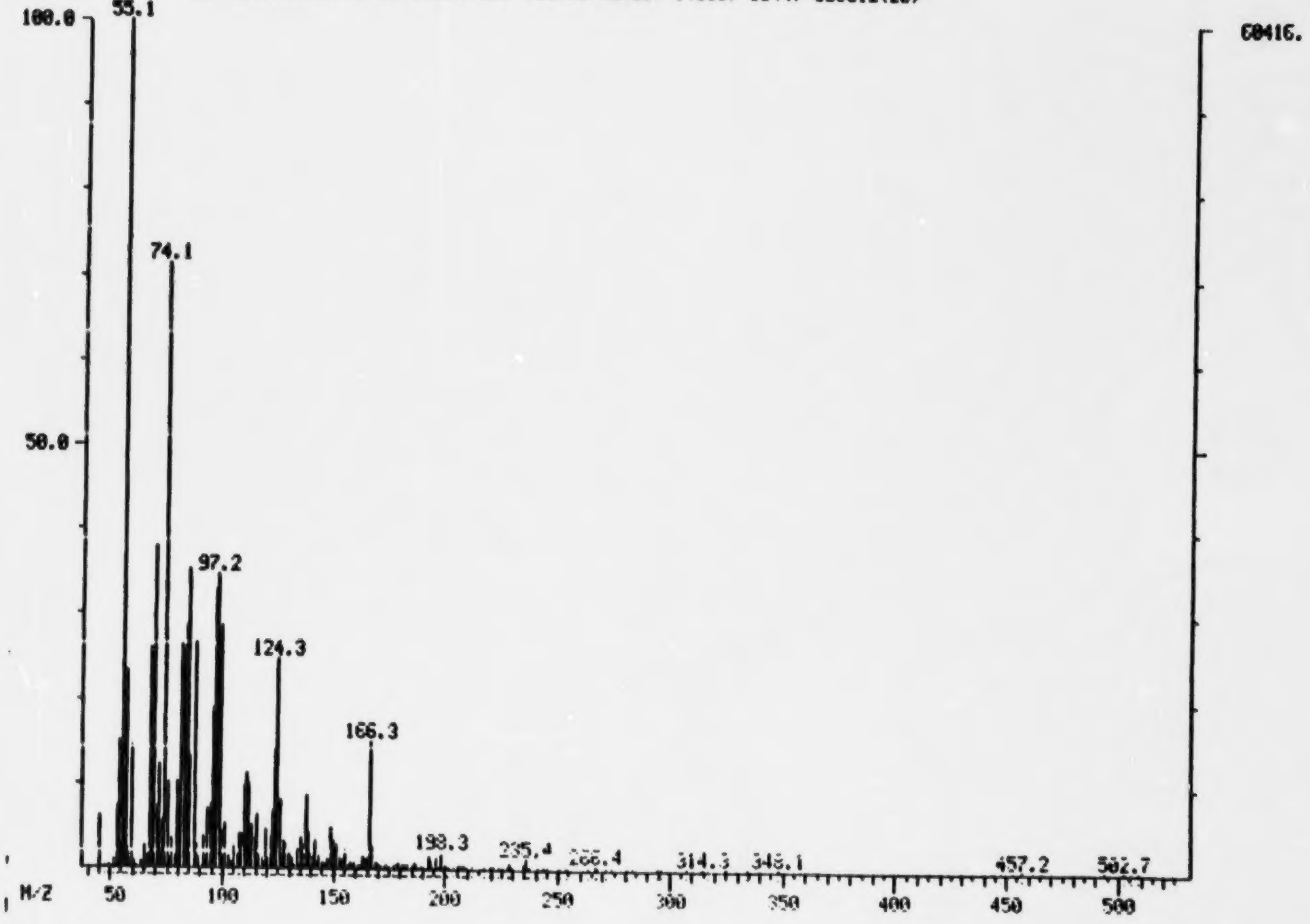


MM5 SAMPLE 3
PEAK 9
COMPARISON LIBRARY MASS SPECTRA

MASS SPECTRUM
05/07/86 9:54:00 + 22:40
SAMPLE: 41702-08-03 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EU
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

DATA: 567814 #1930
CALI: 567810CAL32 #4

BASE M/Z: 55
RIC: 568320.



MM5 SAMPLE 3
PEAK 10
MASS SPECTRUM

```
Library Search                Data: 567814 #1930        Base m/z:    55
05/07/86  9:54:00 + 22:40     Cali: 567810CAL32 #   4   RIC:     468991.
Sample: 41702-08-03 MMS EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

39763 spectra in LIBRARYNB searched for maximum PURITY
   399 matched at least 6 of the 16 largest peaks in the unknown

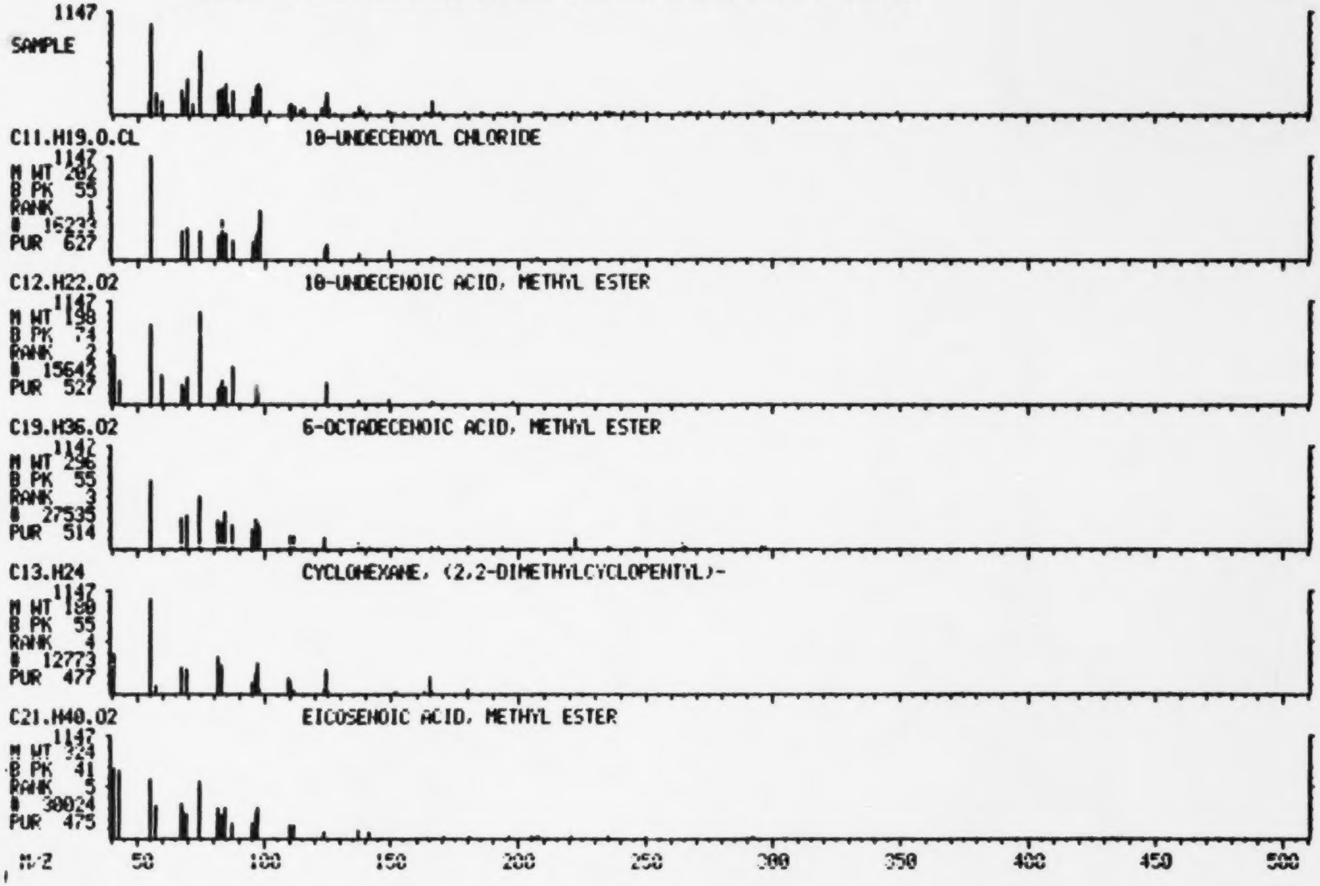
Rank In.       Name
1 16233 10-UNDECENOYL CHLORIDE
2 15642 10-UNDECENOIC ACID, METHYL ESTER
3 27535 6-OCTADECENOIC ACID, METHYL ESTER
4 12773 CYCLOHEXANE, (2,2-DIMETHYLCYCLOPENTYL)-
5 30024 EICOSENOIC ACID, METHYL ESTER
6 27525 10-OCTADECENOIC ACID, METHYL ESTER
7 27358 13,16-OCTADECADIENOIC ACID, METHYL ESTER
8 27349 14,17-OCTADECADIENOIC ACID, METHYL ESTER
9 28996 ETHANOL, 2-(9-OCTADECENYLOXY)-, (Z)-

Rank  Formula                              M.Wt B.Pk   Purity    Fit   RFit
1     C11.H19.O.CL                          202   55      627    939    627
2     C12.H22.O2                            198   74      527    863    573
3     C19.H36.O2                            296   55      514    805    607
4     C13.H24                               180   55      477    820    514
5     C21.H40.O2                            324   41      475    809    571
6     C19.H36.O2                            296   55      470    750    596
7     C19.H34.O2                            294   41      466    777    554
8     C19.H34.O2                            294   41      460    755    517
9     C20.H40.O2                            312   55      457    797    526

Rank     Ret Time      B.P.Int       US.Par.1      US.Par.2        C.A.S. #
1          ___            ___         0.9840          ___         38460-95-6
2          ___            ___         0.7120          ___           111-81-9
3          ___            ___         0.7190          ___         52355-31-4
4          ___            ___         0.6750          ___         61142-23-2
5          ___            ___         0.6110          ___         27070-40-2
6          ___            ___         0.6890          ___         13481-95-3
7          ___            ___         0.7190          ___         56846-99-2
8          ___            ___         0.7160          ___         56554-60-0
9          ___            ___         0.6760          ___          5353-25-3
```

MMS SAMPLE 3
PEAK 10
LIBRARY SEARCH REPORT

LIBRARY SEARCH DATA: 567814 #1930 BASE M/Z: 55
05/07/86 9:54:00 + 22:40 CALI: 567810CAL32 # 4 RIC: 468991.
SAMPLE: 41702-08-03 MM5 EXTRACT 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

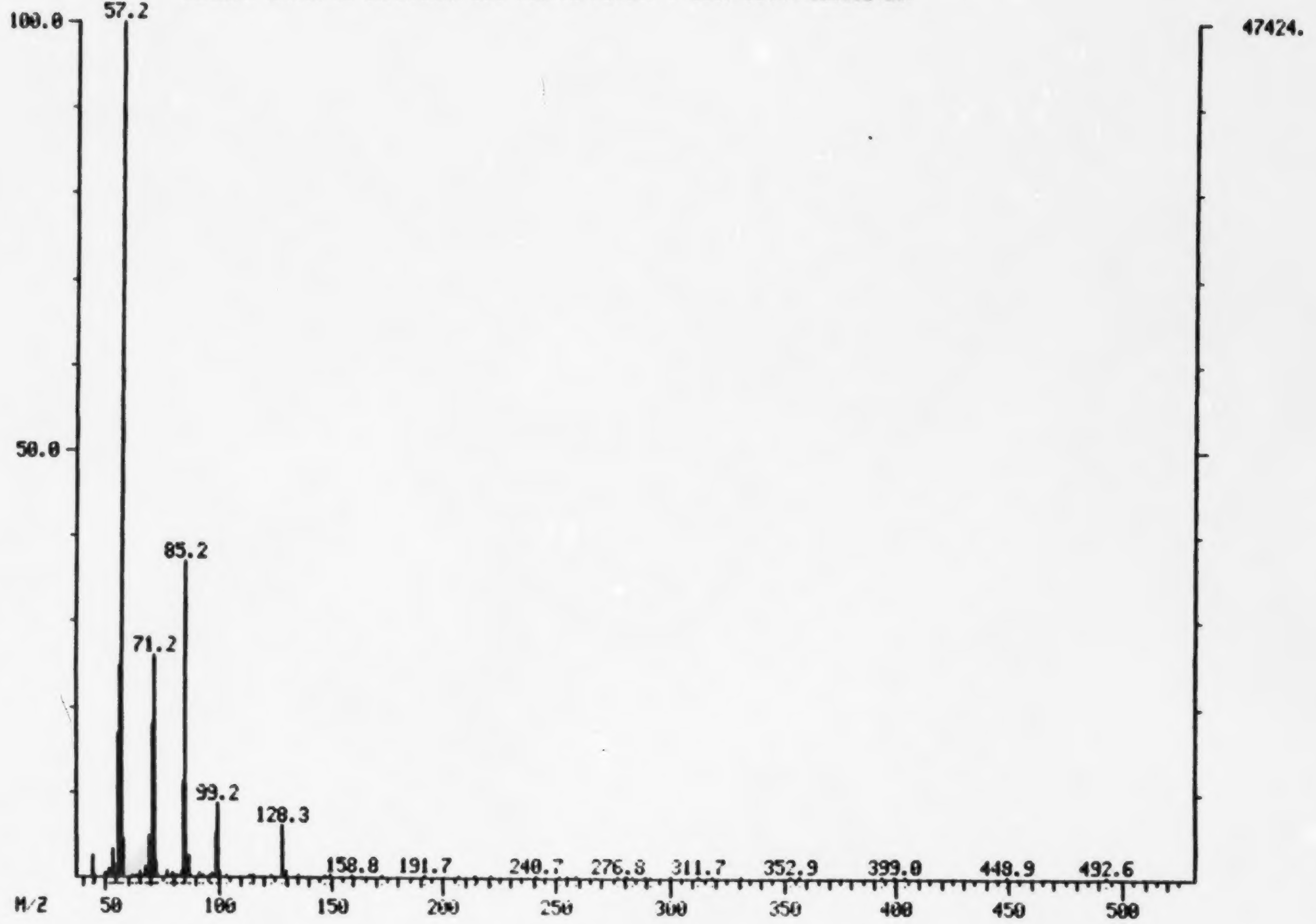


MM5 SAMPLE 3
PEAK 10
COMPARISON LIBRARY MASS SPECTRA

MASS SPECTRUM
05/09/86 10:21:00 + 5:26
SAMPLE: 41702-06-14 COMP WATER EXTPS #1-7 2.0UL SPLITLESS 10-7 @ 1196 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

DATA: 568015 #463
CALI: 568010CAL32 #4

BASE M/Z: 57
RIC: 137728.



COMPOSITE MIBAS SAMPLE
PEAK 1
MASS SPECTRUM

```
Library Search               Data: 568015 # 463          Base m/z:    57
05/09/86 10 21:00 +  5:26    Cal: 568010CAL32 #   4    RIC.     134911.
Sample: 41702-06-14 COMP WATER EXTRS #1-7 2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI,GC/MS 30MX 25MM DB5 #36779 HE(30) I(300) 60(4)-320@12(20)

39763 spectra in LIBRARYNB searched for maximum PURITY
  228 matched at least 6 of the 16 largest peaks in the unknown

Rank In.        Name
1  8509 OCTANE, 2,2,6-TRIMETHYL-
2  8506 OCTANE, 2,5,6-TRIMETHYL-
3 13499 HEPTANE, 2,2,3,4,6,6-HEXAMETHYL-
4 13493 DECANE, 2,5,6-TRIMETHYL-
5  8512 OCTANE, 2,4,6-TRIMETHYL-
6  8502 NONANE, 2,5-DIMETHYL-
7  3924 3(2H)-FURANONE, DIHYDRO-5-ISOPROPYL-
8 13498 HEPTANE, 4-ETHYL-2,2,6,6-TETRAMETHYL-
9 13487 UNDECANE, 5,6-DIMETHYL-

Rank   Formula                                M Wt B Pk    Purity    Fit    RFit
1      C11.H24                                 156   57       649    852     720
2      C11.H24                                 156   57       638    793     765
3      C13.H28                                 184   57       638    807     741
4      C13.H28                                 184   57       608    754     773
5      C11.H24                                 156   57       603    750     786
6      C11.H24                                 156   57       585    726     785
7      C7.H12.O2                               128   57       578    805     670
8      C13.H28                                 184   57       577    776     640
9      C13.H28                                 184   57       575    713     753

Rank     Ret Time      B P Int       US Par 1     US Par 2      C.A.S. #
1         ____          ____          0.2560        ____        62016-28-8
2         ____          ____          0.3180        ____        62016-14-2
3         ____          ____          0.2670        ____        62108-32-1
4         ____          ____          0.3070        ____        62108-23-0
5         ____          ____          0.4160        ____        62016-37-9
6         ____          ____          0.3630        ____        17302-27-1
7         ____          ____          0.6160        ____        34004-69-8
8         ____          ____          0.2140        ____        62108-31-0
9         ____          ____          0.3260        ____        17615-91-7
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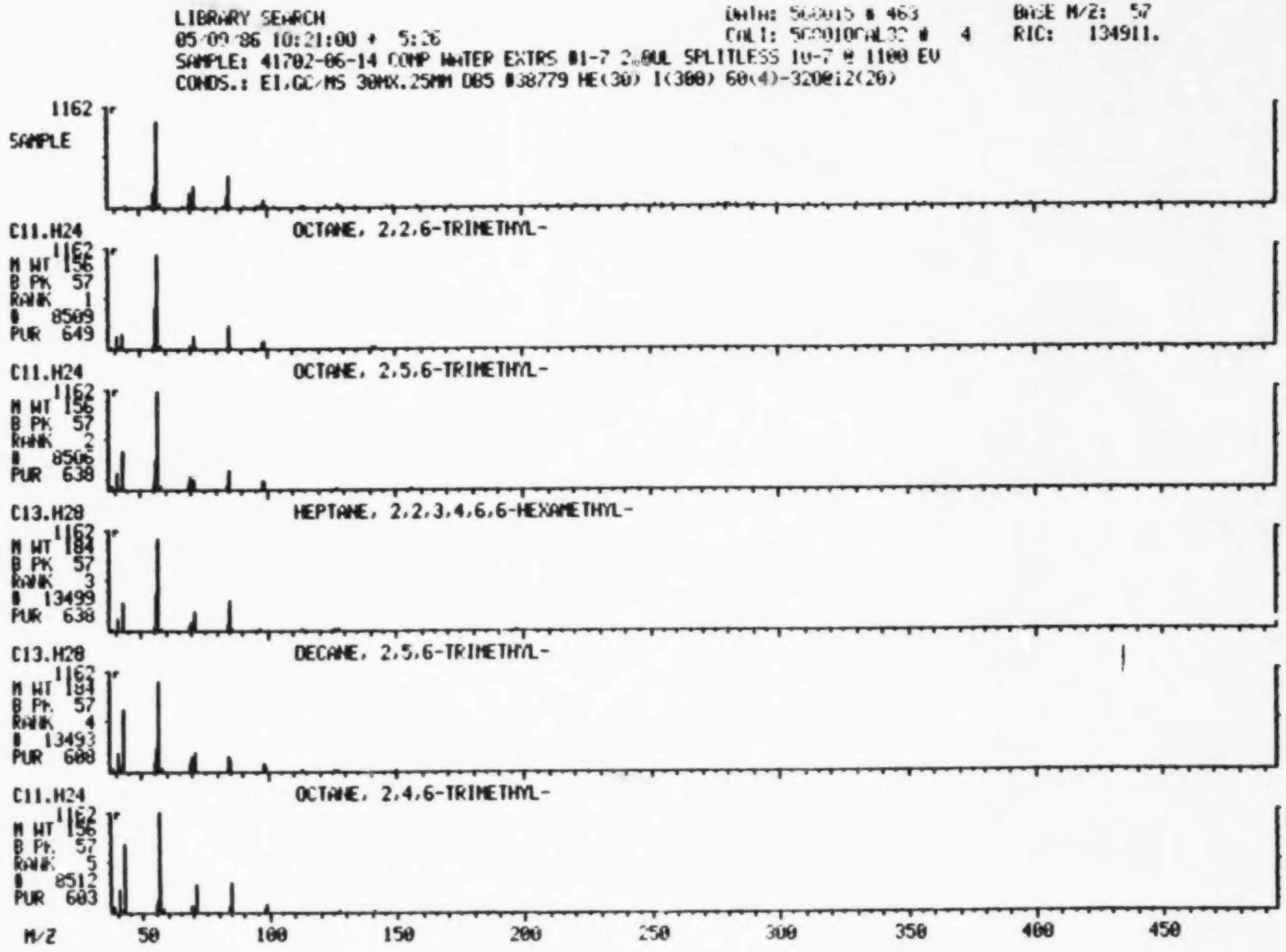
COMPOSITE MIRAS SAMPLE
PEAK 1
LIBRARY SEARCH REPORT



```
LIBRARY SEARCH                                   DATA: 560015 # 463        BASE M/Z:  57
05/09/86 10:21:00 +  5:26                        CALI: 560010CAL32 #   4   RIC:    134911.
SAMPLE: 41702-06-14 COMP WATER EXTRS #1-7 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
```

```
1162
SAMPLE

C11.H24                OCTANE, 2,2,6-TRIMETHYL-
     1162
M WT  156
B PK   57
RANK    1
#    8509
PUR   649

C11.H24                OCTANE, 2,5,6-TRIMETHYL-
     1162
M WT  156
B PK   57
RANK    2
#    8506
PUR   638

C13.H28                HEPTANE, 2,2,3,4,6,6-HEXAMETHYL-
     1162
M WT  184
B PK   57
RANK    3
#   13499
PUR   638

C13.H28                DECANE, 2,5,6-TRIMETHYL-
     1162
M WT  184
B PK   57
RANK    4
#   13493
PUR   608

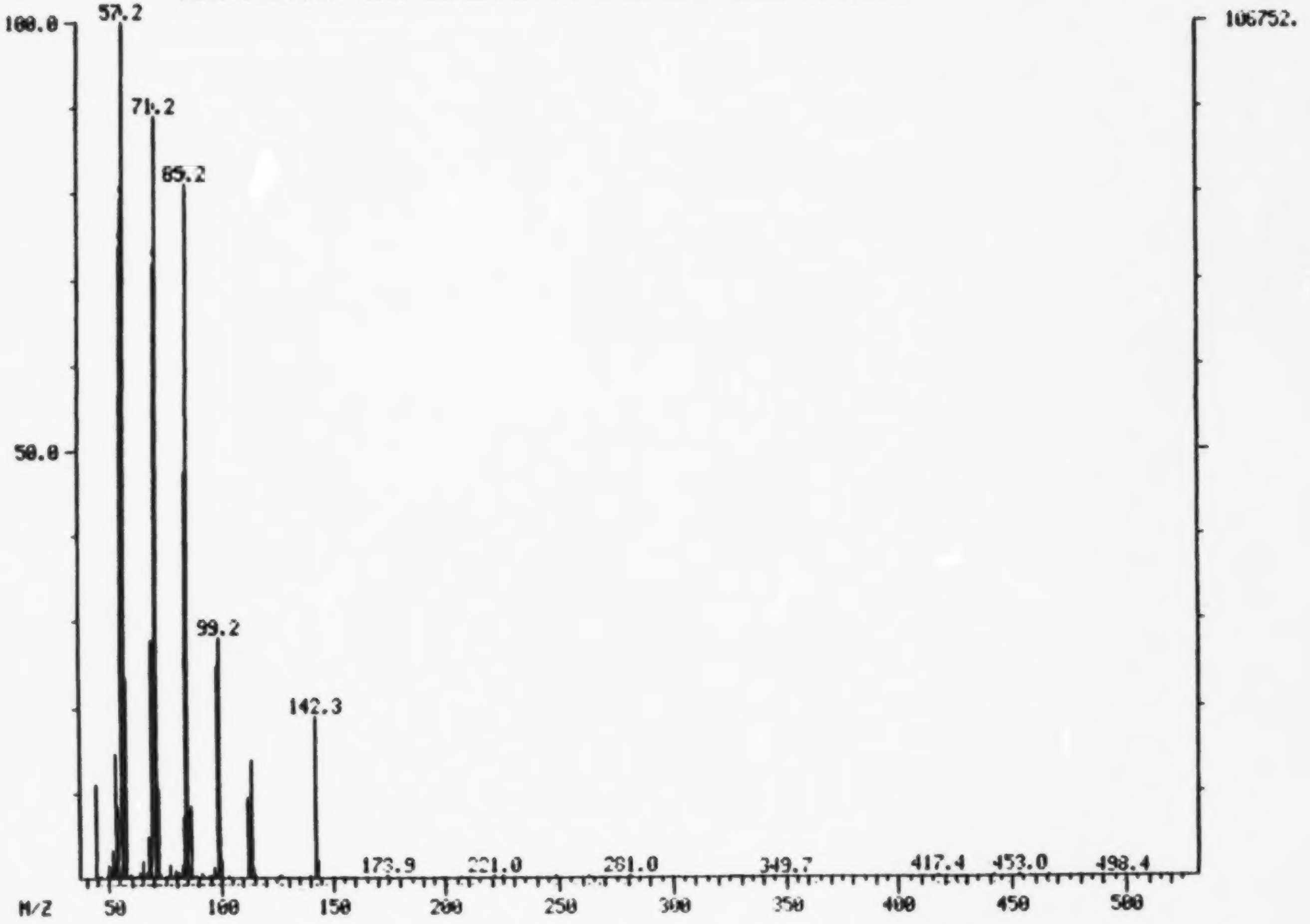
C11.H24                OCTANE, 2,4,6-TRIMETHYL-
     1162
M WT  156
B PK   57
RANK    5
#    8512
PUR   603
```

COMPOSITE MIBAS SAMPLE
PEAK 1
COMPARISON LIBRARY MASS SPECTRA

MASS SPECTRUM
05/09/86 10:21:00 + 7:42
SAMPLE: 41702-06-14 COMP WATER EXTRS 01-7 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

DATA: 560815 L655
CALI: 5C0010CAL32 #4

BASE M/Z: 57
RIC: 833536.



COMPOSITE MIBAS SAMPLE
PEAK 2
MASS SPECTRUM

```
Library Search              Data: 568015 # 655          Base m/z:    57
05/09/86 10:21:00 +  7:42   Cali: 568010CAL32 #   4   RIC      81715:.
Sample: 41702-06-14 COMP WATER EXTRS #1-7 2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI.GC/MS 30MX.25MM DB5 #38779 HE(30) 1(300) 60(4)-320@12(20)

39763 spectra in LIBRARYNB searched for maximum PURITY
  335 matched at least 6 of the 16 largest peaks in the unknown

Rank In.       Name
1  5940 DECANE
2 13493 DECANE, 2,5,6-TRIMETHYL-
3 13458 UNDECANE, 3,6-DIMETHYL-
4 13483 UNDECANE, 4,6-DIMETHYL-
5 13487 UNDECANE, 5,6-DIMETHYL-
6  8501 NONANE, 4,5-DIMETHYL-
7  8502 NONANE, 2,5-DIMETHYL-
8  8506 OCTANE, 2,5,6-TRIMETHYL-
9 16018 1-TRIDECANOL

Rank   Formula                                  M.Wt  B.Pk   Purity   Fit   RFit
1      C10 H22                                   142   57      645    745    611
2      C13 H28                                   184   57      643    784    745
3      C13 H28                                   184   57      620    740    723
4      C13 H28                                   184   57      615    714    740
5      C13 H28                                   184   57      613    731    722
6      C11 H24                                   156   43      611    750    725
7      C11 H24                                   156   57      609    726    718
8      C11 H24                                   156   57      608    768    678
9      C13 H28 O                                 200   57      594    757    680

Rank     Ret Time      B.F In.      US Par 1     US Par 2        C.A.S #
1          ____         ____         0.4580        ____          124-18-5
2          ____         ____         0.3070        ____        62108-23-0
3          ____         ____         0.3240        ____        17301-28-9
4          ____         ____         0.3410        ____        17312-82-2
5          ____         ____         0.3260        ____        17615-91-7
6          ____         ____         0.4210        ____        17302-23-7
7          ____         ____         0.3630        ____        17302-27-1
8          ____         ____         0.3180        ____        62016-14-2
9          ____         ____         0.7210        ____          112-70-9
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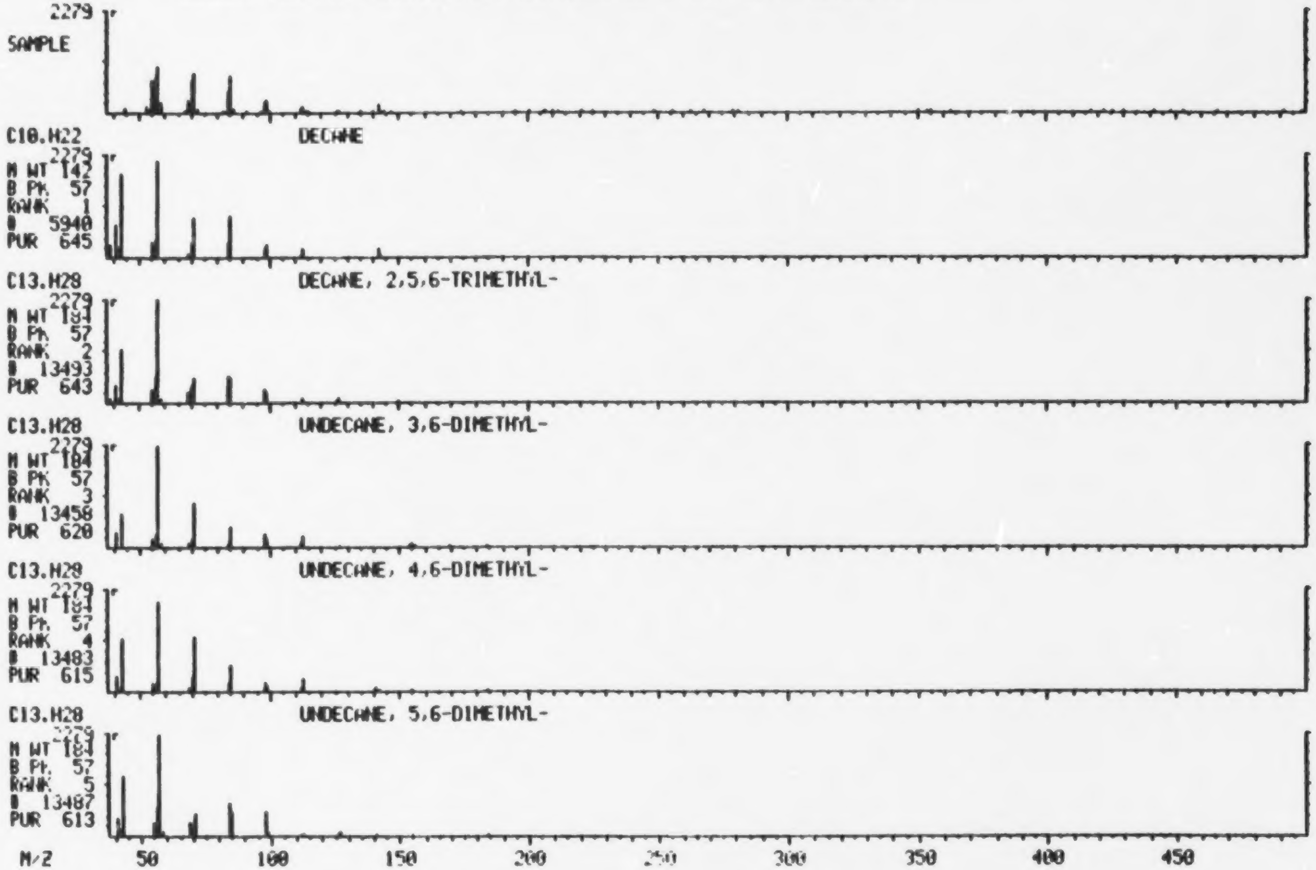
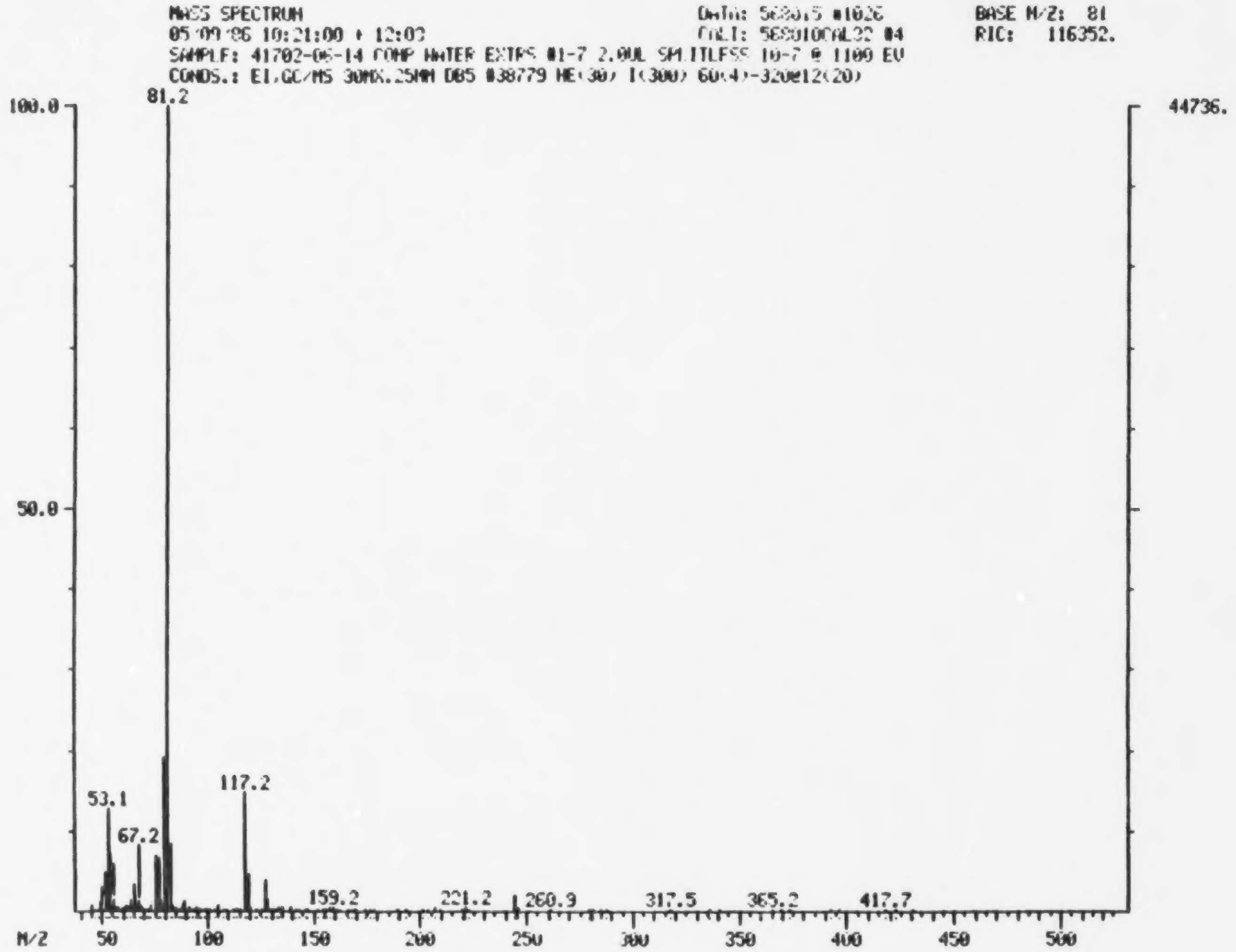
COMPOSITE MIBAS SAMPLE
PEAK 2
LIBRARY SEARCH REPORT

```
LIBRARY SEARCH                                        DATA: 568015 # 655      BASE M/Z:  57
05/09/86 10:21:00 + 7:42                              CALI: 569010CAL32 #  4  RIC:   817151.
SAMPLE: 41702-06-14 COMP WATER EXTPS #1-7 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
```



COMPOSITE MIBAS SAMPLE
PEAK 2
COMPARISON LIBRARY MASS SPECTRA



MASS SPECTRUM
05/09/86 10:21:00 + 12:03
SAMPLE: 41702-06-14 COMP WATER EXTRS #1-7 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

DATA: SE8015 #1026
CALI: SE8010CAL22 #4

BASE M/Z: 81
RIC: 116352.

COMPOSITE MIBAS SAMPLE
PEAK 3
MASS SPECTRUM

```
                                   Data: 568015 #1026         Base m/z:    81
Library Search
05/09/86 10:21:00 + 12:03          Cali: 568010CAL32 #   4    RIC       105855
Sample: 41702-06-14 COMP WATER EXTRS #1-7 2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI,GC/MS 30MX.25MM DB5 #32779 HE(30) I(300) 60(4)-320@12(20)

39763 spectra in LIBRARYNB searched for maximum PURITY
  337 matched at least 5 of the 16 largest peaks in the unknown

Rank In.       Name
1   9246 CYCLOHEXENE, 3-BROMO-
2   7738 CYCLOHEXANE, 1,2-DICHLORO-, CIS-
3  15443 CYCLOHEXANE, 1-BROMO-2-CHLORO-, CIS-
4   2720 CYCLOPROPANE, 1-CHLORO-2-(1-PROPENYL)-
5   2719 CYCLOPROPANE, 1-CHLORO-2-ETHENYL-1-METHYL-
6   2718 2,4-HEXADIENE, 1-CHLORO-
7   7739 CYCLOHEXANE, 1,4-DICHLORO-, CIS-
8   2715 CYCLOHEXENE, 3-CHLORO-
9   3379 ETHANONE, 1-(1-METHYL-2-CYCLOPENT[illegible]-1-YL)-

                                               M.Wt  B.Pk   Purity    Fit    RFit
Rank   Formula
1      C6.H9.BR                                 160    81      580    804     632
2      C6.H10.CL2                               152    81      569    804     592
3      C6.H10.CL.BR                             196    81      562    803     696
4      C6.H9.CL                                 116    81      547    882     595
5      C6.H9.CL                                 116    81      541    892     585
6      C6.H9.CL                                 116    81      533    836     583
7      C6.H10.CL2                               152    81      525    770     610
8      C6.H9.CL                                 116    81      524    845     557
9      C8.H12.O                                 124    81      521    863     587

                                        US Par 1       US Par 2        C.A.S. #
Rank      Ret Time        B.P.Int          0.5530                       1521-51-3
1          ____            ____            0.3380        ____         10498-35-8
2          ____            ____            0.2090        ____         51422-75-4
3          ____            ____            0.7430        ____         74752-94-6
4          ____            ____            0.7110        ____         62337-93-3
5          ____            ____            0.5730        ____         34632-89-8
6          ____            ____            0.4090        ____         16749-11-4
7          ____            ____            0.7270        ____          2441-97-6
8          ____            ____            0.4650        ____         68752-16-9
9          ____            ____                          ____
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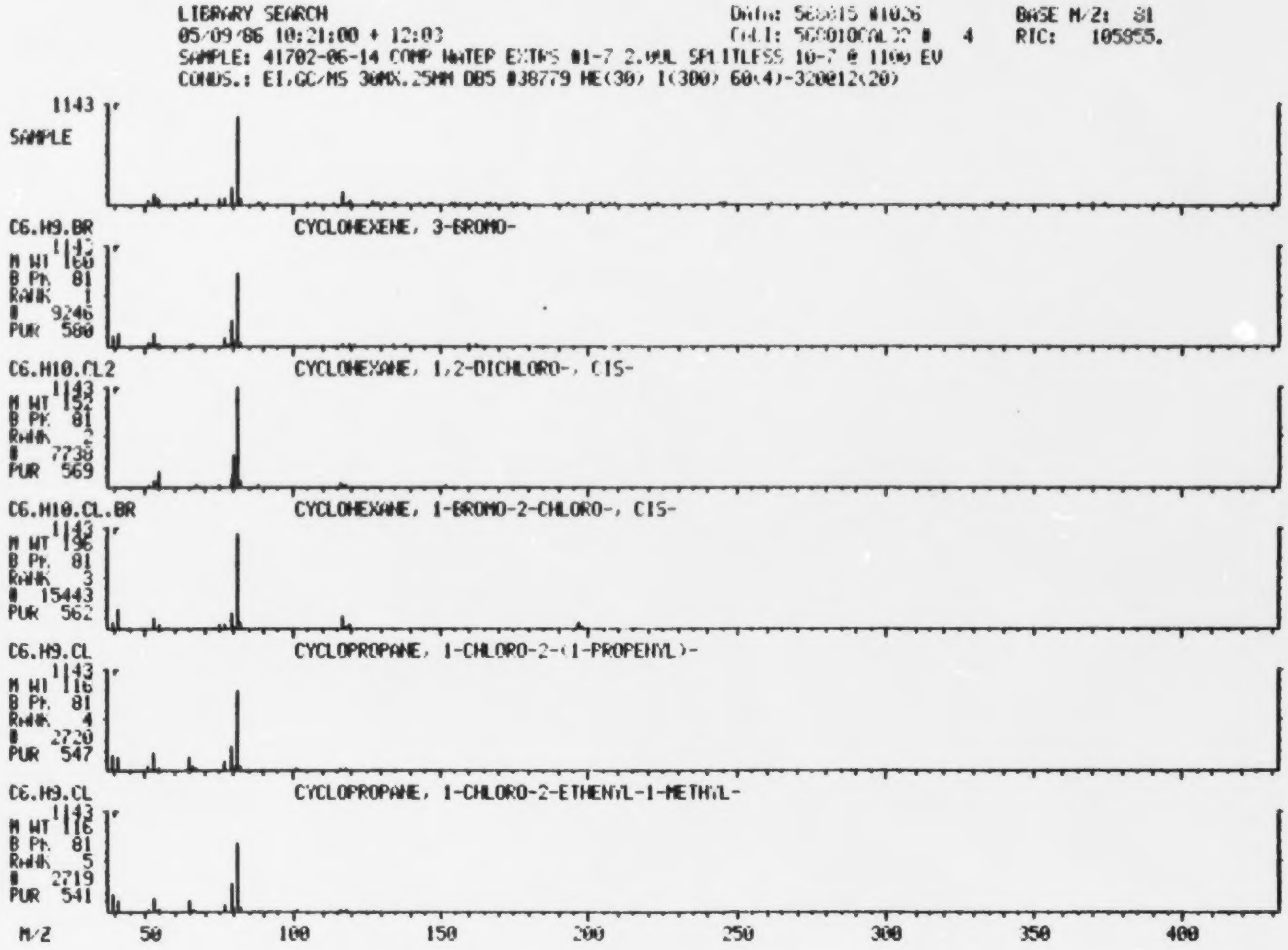
COMPOSITE MIBAS SAMPLE
PEAK 3
LIBRARY SEARCH REPORT



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LIBRARY SEARCH                                  DATA: 560015 #1026        BASE M/Z:  81
05/09/86 10:21:00 + 12:03                       CALI: 560010CAL32 #   4   RIC:    105955.
SAMPLE: 41702-06-14 COMP WATER EXTRS #1-7 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)
```

```
1143
SAMPLE

C6.H9.BR          CYCLOHEXENE, 3-BROMO-
    1143
M WT 160
B PK  81
RANK   1
#   9246
PUR  580

C6.H10.CL2        CYCLOHEXANE, 1,2-DICHLORO-, CIS-
    1143
M WT 152
B PK  81
RANK   2
#   7738
PUR  569

C6.H10.CL.BR      CYCLOHEXANE, 1-BROMO-2-CHLORO-, CIS-
    1143
M WT 196
B PK  81
RANK   3
#  15443
PUR  562

C6.H9.CL          CYCLOPROPANE, 1-CHLORO-2-(1-PROPENYL)-
    1143
M WT 116
B PK  81
RANK   4
#   2720
PUR  547

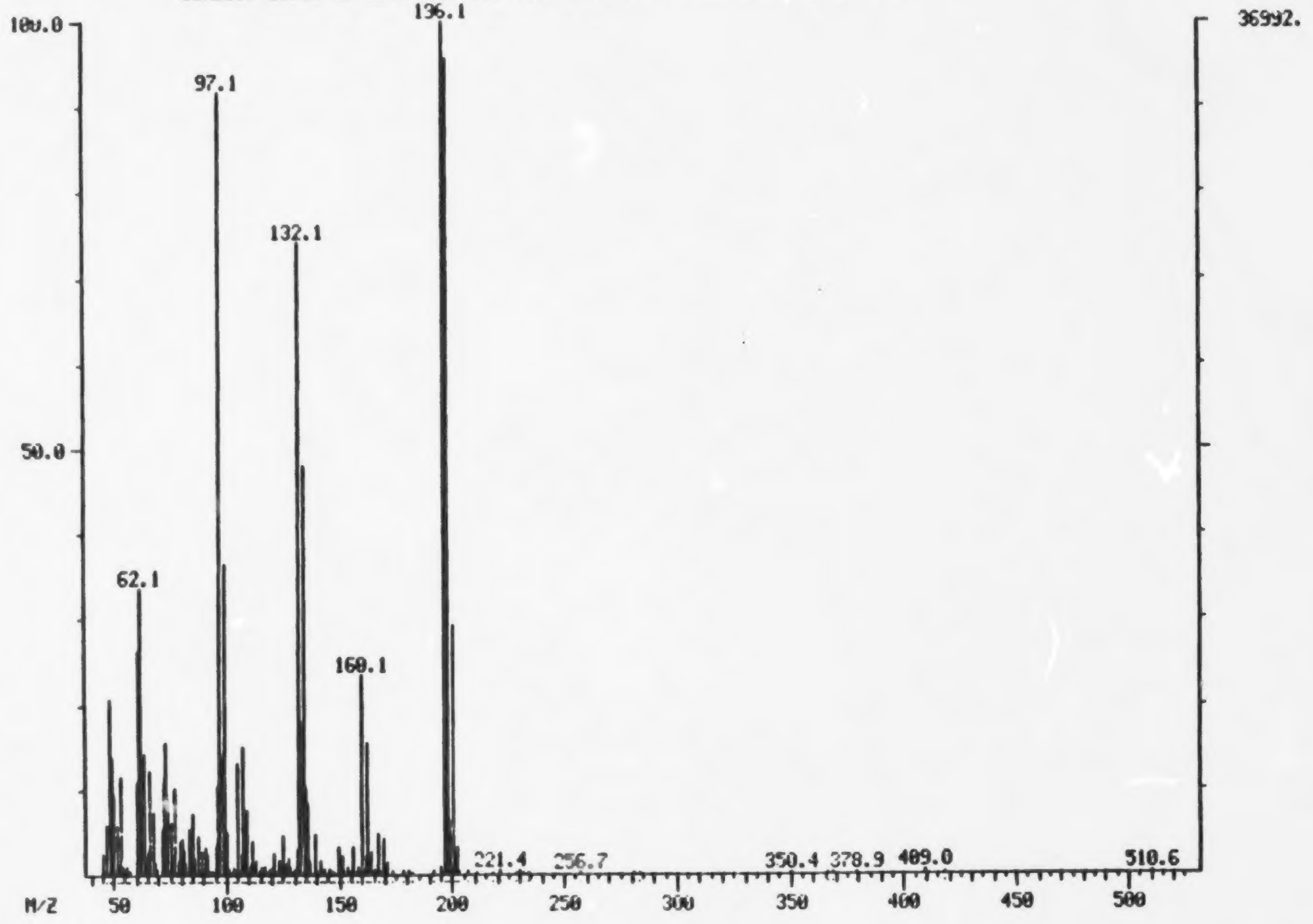
C6.H9.CL          CYCLOPROPANE, 1-CHLORO-2-ETHENYL-1-METHYL-
    1143
M WT 116
B PK  81
RANK   5
#   2719
PUR  541
```

COMPOSITE MIBAS SAMPLE
PEAK 3
COMPARISON LIBRARY MASS SPECTRA

MASS SPECTRUM
05/09/86 10:21:00 + 13:24
SAMPLE: 41702-06-14 COMP WATER EXTRS #1-7 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

DATA: 568015 #1141
CALI: 568010CAL32 #4

BASE M/Z: 196
RIC: 377856.



COMPOSITE MIBAS SAMPLE
PEAK 4
MASS SPECTRUM

```
Library Search              Data: 568015 #1141         Base m/z:   196
05/09/86 10:21:00 + 13:24   Cali: 568010CAL32 #   4    RIC:     335359
Sample  41702-06-14 COMP WATER EXTRS #1-7 2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI.GC/MS 30MX 25MM DB5 #35779 HF(30) I(300) 60(4)-320@12(20)

39763 spectra in LIBRARY NB searched for maximum PURITY
  155 matched at least 4 of the 16 largest peaks in the unknown

Rank In.       Name
1 15447 PHENOL, 2,4,6-TRICHLORO-
2 15450 PHENOL, 2,3,4-TRICHLORO-
3 15446 PHENOL, 2,4,5-TRICHLORO-
4 19611 BENZENE, 1,2,4-TRICHLORO-5-ETHOXY-
5 15449 PHENOL, 2,3,6-TRICHLORO-
6 24803 PROPANOIC ACID, 2-(2,4,5-TRICHLOROPHENOXY)-
7 15285 NAPHTHALENE, 1,8-DICHLORO-
8 15283 NAPHTHALENE, 1,4-DICHLORO-
9 15282 NAPHTHALENE, 1,5-DICHLORO-

Rank  Formula                                M.Wt B.Pk   Purity    Fit    RFit
1     C6 H3 O CL3                             196  196      810    967     814
2     C6 H3 O CL3                             196  196      806    964     815
3     C6 H3 O CL3                             196  196      729    991     729
4     C8 H7 O CL3                             224  196      647    807     745
5     C6 H3 O CL3                             196  196      635    875     680
6     C9 H7 O3 CL3                            268  196      452    669     659
7     C10 H6 CL2                              196  196      418    848     449
8     C10 H6 CL2                              196  196      404    835     435
9     C10 H6 CL2                              196  196      401    826     432

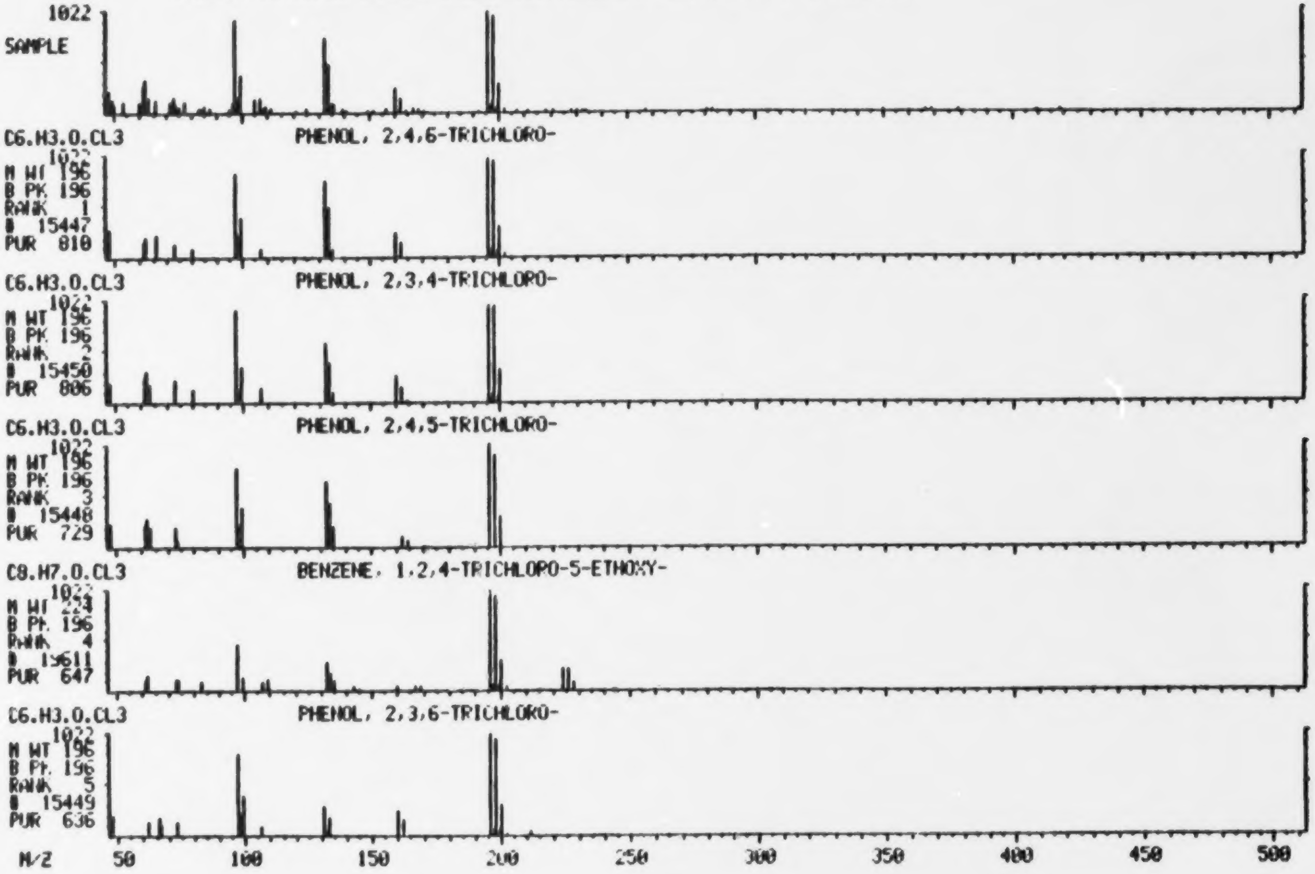
Rank     Ret Time       B F Int      US Par 1      US Par 2         C.A.S. #
1                                     0.6510                         88-06-2
2         ____          ____          0.6900        ____          15950-66-0
3         ____          ____          0.6610        ____             95-95-4
4         ____          ____          0.6550        ____           6851-44-1
5         ____          ____          0.5880        ____            933-75-5
6         ____          ____          0.7281        ____             93-72-1
7         ____          ____          0.3780        ____           2050-74-0
8         ____          ____          0.3410        ____           1825-31-6
9         ____          ____          0.3960        ____           1825-30-5
          ____          ____                        ____
```

COMPOSITE MIBAS SAMPLE
PEAK 4
LIBRARY SEARCH REPORT

LIBRARY SEARCH
05/09/86 10:21:00 + 13:24
SAMPLE: 41702-06-14 COMP WATER EXTRS #1-7 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

DATA: 568015 #1141
CALI: 568010CAL32 # 4

BASE M/Z: 196
RIC: 335359.

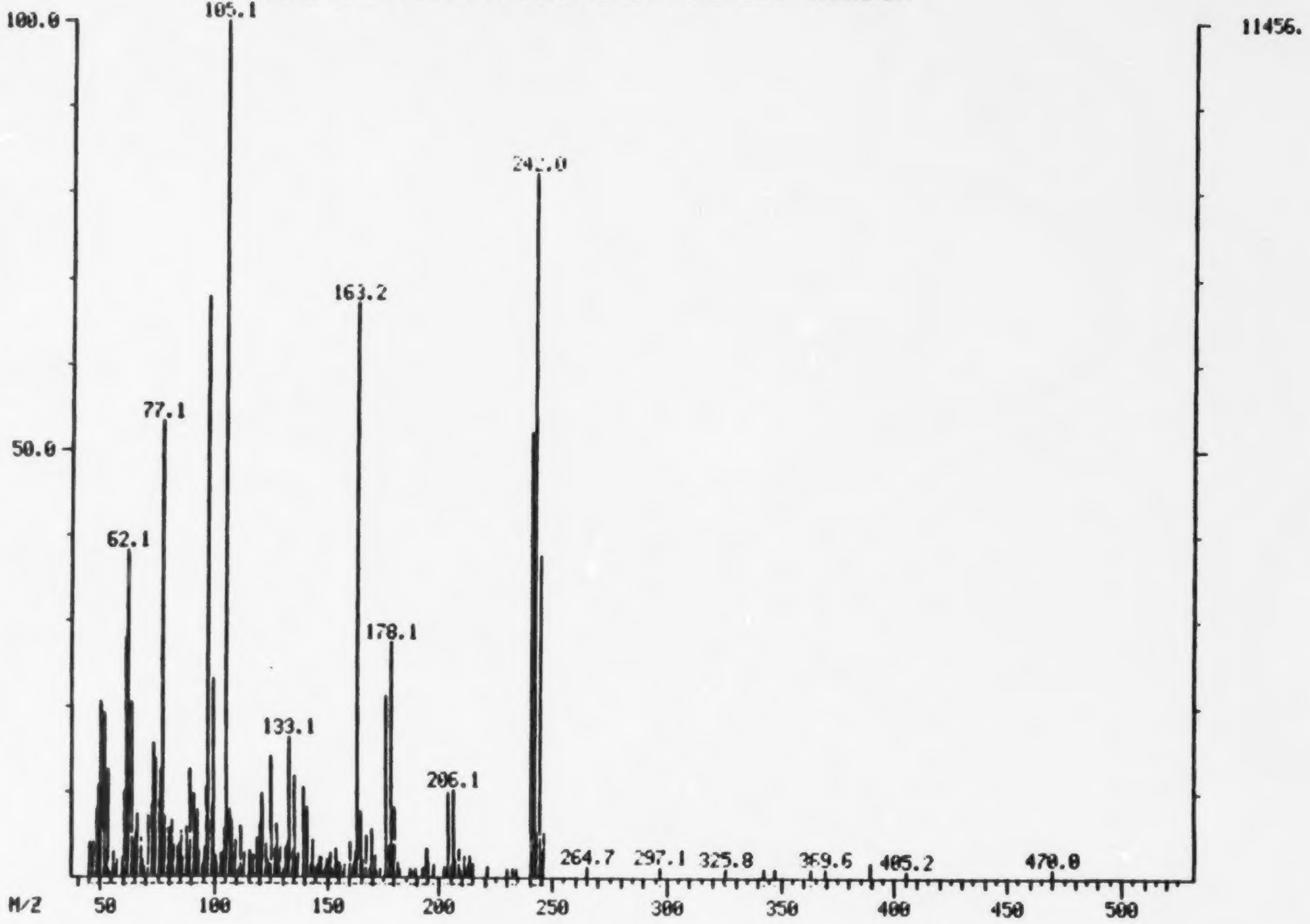


COMPOSITE MIBAS SAMPLE
PEAK 4
COMPARISON LIBRARY MASS SPECTRA

MASS SPECTRUM
05/09/86 10:21:00 + 14:30
SAMPLE: 41702-0--14 COMP WATER EXTRS #1-7 2.0UL SPLITLESS 10-7 @ 1100 EU
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

DATA: 560015 #1246
CALI: 560010CAL32 #4

BASE M/Z: 105
RIC: 145920.



COMPOSITE MIBAS SAMPLE
PEAK 5
MASS SPECTRUM

```
Library Search              Data: 568015 #1246          Base m/z:  105
05/09/86 10:21:00 + 14:38   Cali: 568010CAL32 #   4    RIC:      118271.
Sample: 41702-06-14 COMP WATER EXTRS #1-7 2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20

39763 spectra in LIBRARY NB searched for maximum PURITY
  110 matched at least 4 of the 16 largest peaks in the unknown

Rank In.      Name
1 21620 PHENOL, 4-BROMO-2,5-DICHLORO-
2 21786 2-PROPEN-1-ONE, 1-(2-CHLOROPHENYL)-3-PHENYL-
3 21675 1-BENZOPYRYLIUM, 2-(4-CHLOROPHENYL)-
4 21612 THIOPHENE, 2,3-DIBROMO-
5 21553 BENZOIC ACID, 4-BENZOYL-, METHYL ESTER
6 21785 2-PROPEN-1-ONE, 3-(4-CHLOROPHENYL)-1-PHENYL-
7 21547 BENZOIC ACID, 2-BENZOYL-, METHYL ESTER
8 25118 GERMACYCLOUNDECANE, 1,1-DIETHYL-
9 21613 THIOPHENE, 2,5-DIBROMO-

Rank  Formula                              M.Wt  B.Pk   Purity   Fit   RFit
1     C6.H3 O CL2 BR                        240   242      503   974    515
2     C15 H11 O CL                          242   105      336   755    364
3     C15 H10 O CL                          241   240      313   771    342
4     C4.H2 S BR2                           240   242      306   733    413
5     C15 H12 O3                            240   105      290   864    329
6     C15 H11 O CL                          242   105      286   674    356
7     C15 H12 O3                            240   105      275   776    339
8     C14 H30 GE                            272   103      256   568    409
9     C4 H2 S BR2                           240    82      229   568    377

Rank    Ret Time     B P Int     US Par 1    US Par 2       C.A.S. #
1         ____         ____       0.6210       ____        1940-42-7
2         ____         ____       0.7170       ____       20426-47-5
3         ____         ____       0.4890       ____       49858-91-5
4         ____         ____       0.4190       ____        3140-93-0
5         ____         ____       0.2830       ____        6158-54-9
6         ____         ____       0.4620       ____         956-04-7
7         ____         ____       0.6380       ____         606-28-0
8         ____         ____       0.6790       ____       56438-29-0
9         ____         ____       0.6940       ____        3141-27-3
```

COMPOSITE MIRAS SAMPLE
PEAK 5
LIBRARY SEARCH REPORT

```
LIBRARY SEARCH                                            DATA: 560015 #1246          BASE M/Z: 105
05/09/86 10:21:00 + 14:30                                 CALI: 560010CAL32 #   4     RIC:    118271.
SAMPLE: 41702-06-14 COMP WATER EXTRS #1-7 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) 1(300) 60(4)-320@12(20)
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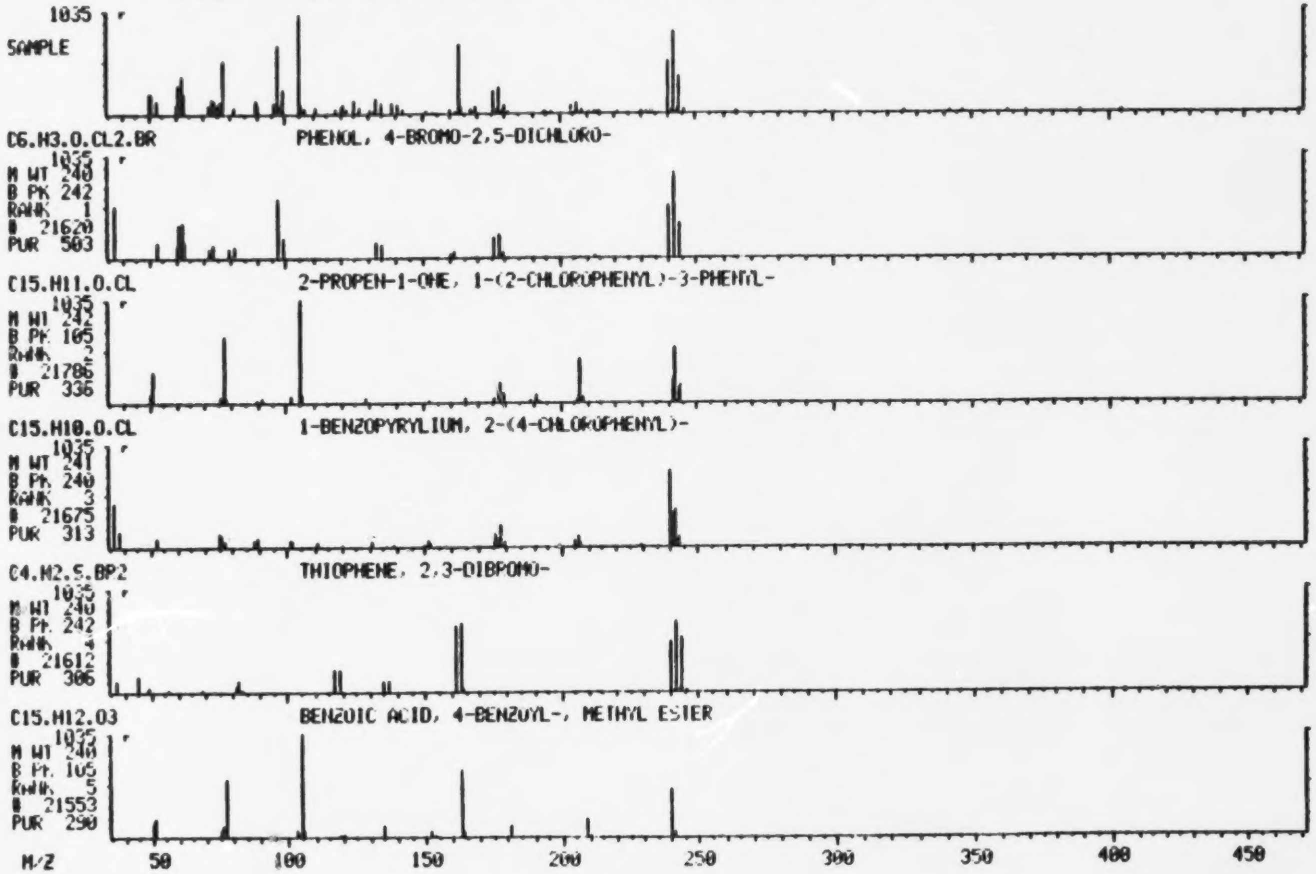


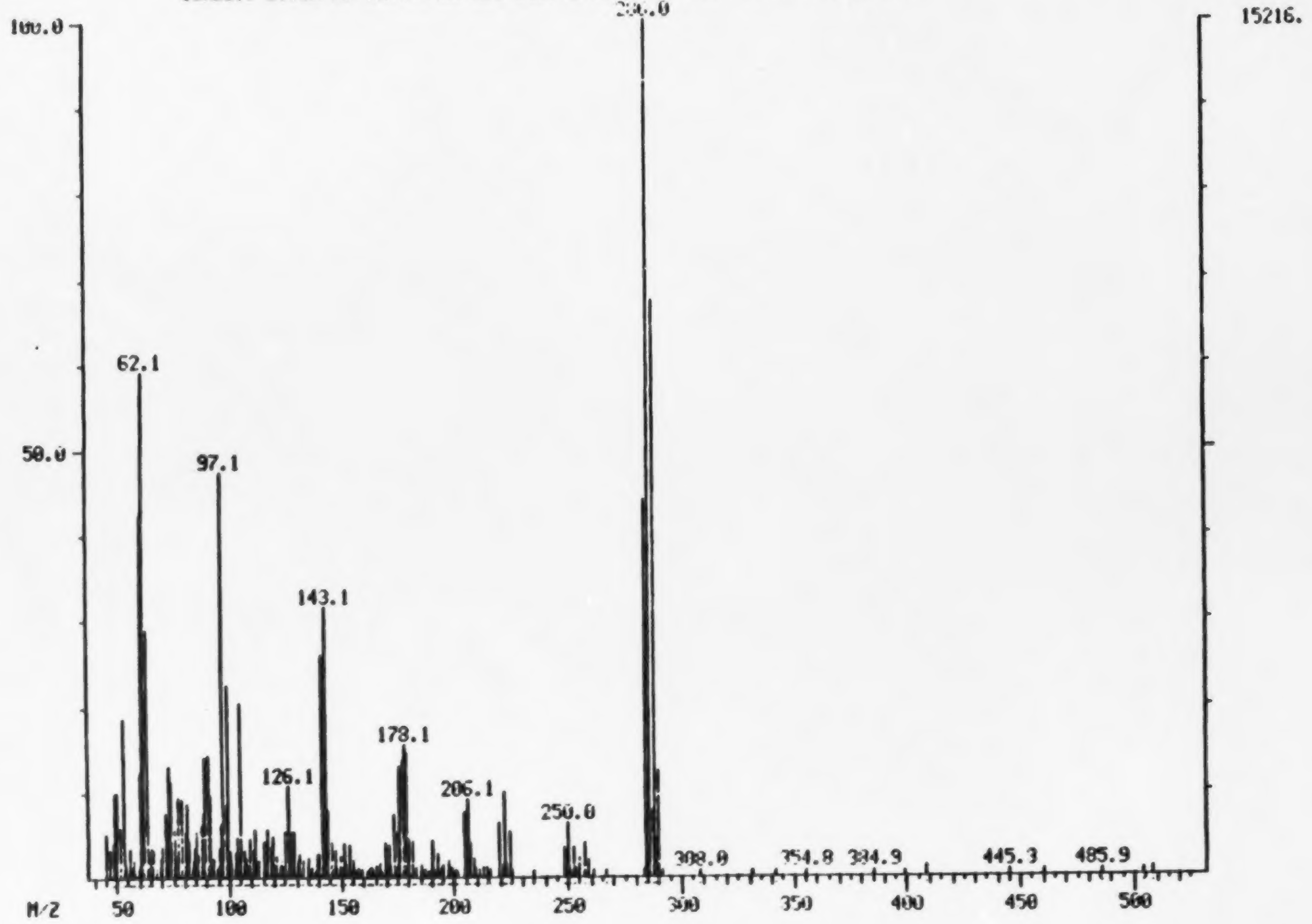
```
SAMPLE
C6.H3.O.CL2.BR     PHENOL, 4-BROMO-2,5-DICHLORO-
M WT 240  B PK 242  RANK 1  # 21620  PUR 503
C15.H11.O.CL       2-PROPEN-1-ONE, 1-(2-CHLOROPHENYL)-3-PHENYL-
M WT 242  B PK 105  RANK 2  # 21786  PUR 336
C15.H10.O.CL       1-BENZOPYRYLIUM, 2-(4-CHLOROPHENYL)-
M WT 241  B PK 240  RANK 3  # 21675  PUR 313
C4.H2.S.BR2        THIOPHENE, 2,3-DIBROMO-
M WT 240  B PK 242  RANK 4  # 21612  PUR 306
C15.H12.O3         BENZOIC ACID, 4-BENZOYL-, METHYL ESTER
M WT 240  B PK 105  RANK 5  # 21553  PUR 290
```

COMPOSITE MIBAS SAMPLE
PEAK 5
COMPARISON LIBRARY MASS SPECTRA

MASS SPECTRUM
05/09/86 10:21:00 + 15:46
SAMPLE: 41702-06-14 COMP WATER EXTRS #1-7 2.0UL SPLITLESS 10-7 @ 1100 EU
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I:300/ 60(4)-320@12(20)

DATA: 5C0015 #1342
CALI: 5C0910CAL32 #4

BASE M/Z: 286
RIC: 178432.



COMPOSITE MIBAS SAMPLE
PEAK 6
MASS SPECTRUM

```
Library Search              Data: 568015 #1342          Base m/z:   286
05/09/86 10:21:00 + 15:46   Cali: 568010CAL32 #   4     RIC:     145407.
Sample: 41702-06-14 COMP WATER EXTRS #1-7 2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI, GC MS 30MX.25MM DB5 #38779 HE(30) 1(300) 60(4)-320@12(20

39763 spectra in LIBRARYNB searched for maximum PURITY
  375 matched at least 3 of the 16 largest peaks in the unknown

Rank In        Name
1 26266 NAPHTHALENE, 1,4-DIBROMO-
2 26514 DIBENZO[B,E][1,4]DIOXIN, 1,2,4-TRICHLORO-
3 26516 DISULFIDE, BIS(4-CHLOROPHENYL)
4 26196 BENZENE, HEXACHLORO-
5 26507 SELENOLO[2,3-B][1]BENZOSELENOPHENE
6 26508 SELENOLO[3,2-B][1]BENZOSELENOPHENE
7 26621 BENZO[C]THIOPHENE, 1,3-DIPHENYL-
8 26197 1,3-CYCLOPENTADIENE, 1,2,3,4-TETRACHLORO-5-(DICHLOROMETHYLENE)-
9 26308 METHANONE, (4-CHLOROPHENYL)(2,4-DICHLOROPHENYL)-

Rank   Formula                                     M.Wt  B.Pk    Purity     Fit    RFit
1      C10 H6 BR2                                   284   126       465     816     474
2      C12 H5 O2 CL3                                286   286       360     753     412
3      C12 H8 S2 CL2                                286   286       340     783     430
4      C6 CL6                                       282   284       330     617     480
5      C10 H6 SE2                                   286   286       332     609     495
6      C10 H6 SE2                                   286   286       324     599     497
7      C20 H14 S                                    286   286       303     582     388
8      C6 CL6                                       282   284       295     564     431
9      C13 H7 O CL3                                 284   173       294     592     350

Rank     Ret.Time      B.P.Int      US.Par.1     US.Par.2      C.A.S. #
1         ____          ____         0.8250        ____          83-53-4
2         ____          ____         0.7140        ____       39227-58-2
3         ____          ____         0.5420        ____        1142-19-4
4         ____          ____         0.6910        ____         118-74-1
5         ____          ____         0.4670        ____       40197-97-5
6         ____          ____         0.4650        ____       40198-00-3
7         ____          ____         0.5270        ____       16587-39-6
8         ____          ____         0.6410        ____        6317-25-5
9         ____          ____         0.6940        ____       33146-57-5
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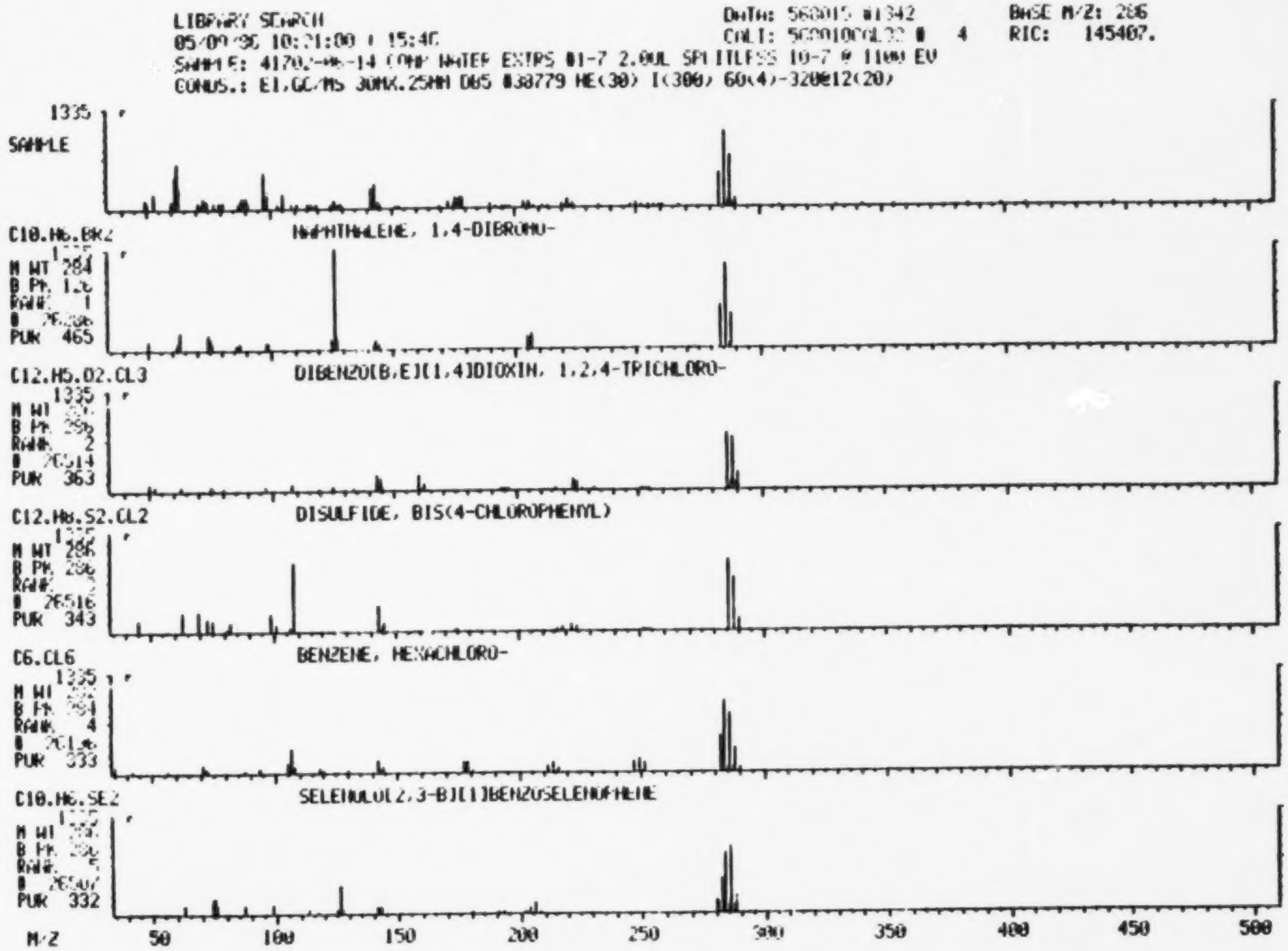
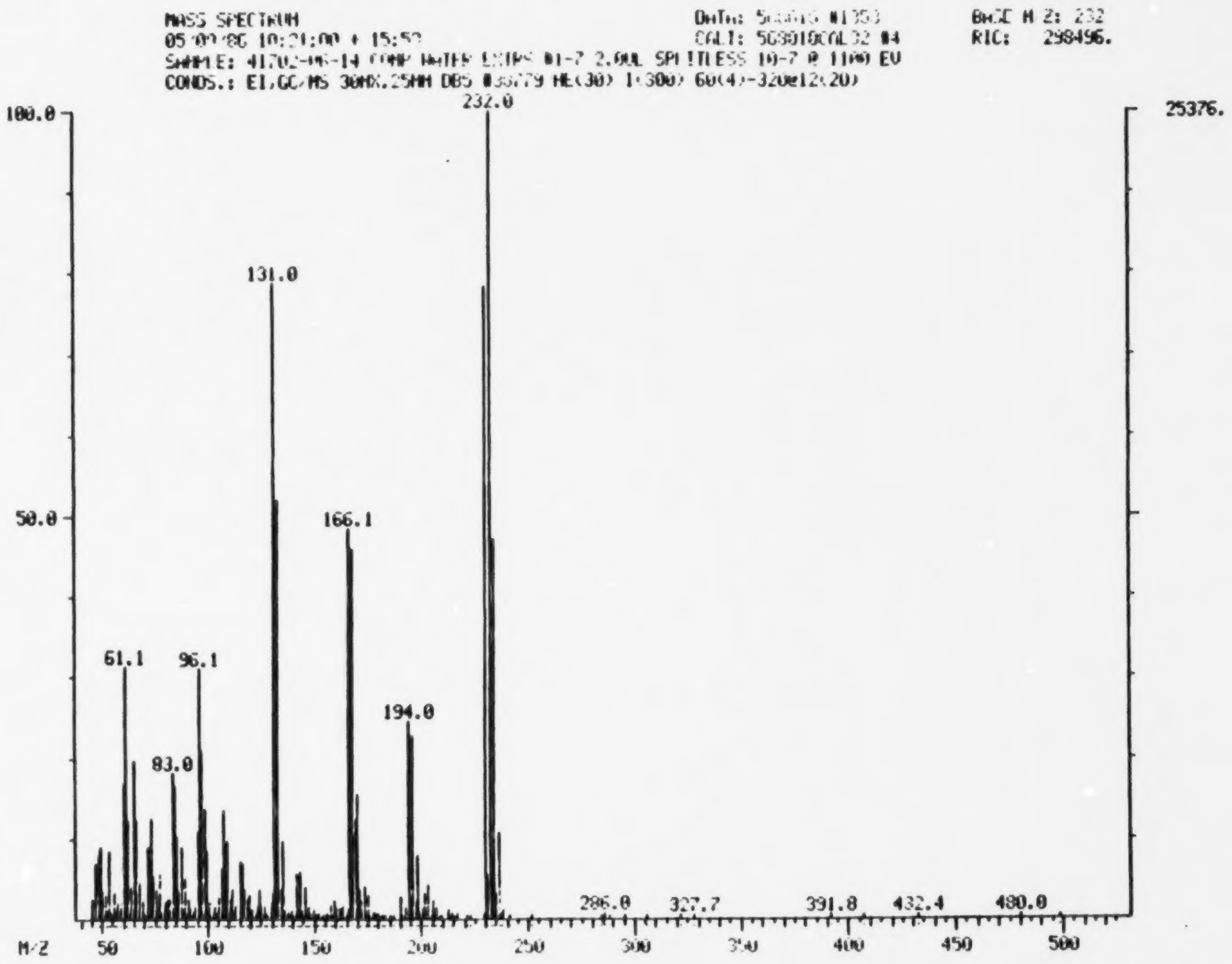
COMPOSITE MIBAS SAMPLE
PEAK 6
LIBRARY SEARCH REPORT



COMPOSITE MIBAS SAMPLE
PEAK 6
COMPARISON LIBRARY MASS SPECTRA



COMPOSITE MIBAC SAMPLE
PEAK 7
MASS SPECTRUM

```
Library Search                 Data: 568015 #1393        Base m/z:  232
05/09/86 10:21:00 + 15:53      Cali: 568010CAL32 #   4   RIC:     253951.
Sample: 41702-06-14 COMP WATER EXTRS #1-7 2.0UL SPLITLESS 10-7 @ 1105 EV
Conds : EI GC/MS 30MX .25MM DB5 #38779 HE(30) I/300/ 60(4)-320@12(20)

39763 spectra in LIBRARY NB searched for maximum PURITY
  139 matched at least 4 of the 10 largest peaks in the unknown

Rank  In        Name
1 20420 PHENOL, 2,3,4,5-TETRACHLORO-
2 20419 PHENOL, 2,3,5,6-TETRACHLORO-
3 20418 PHENOL, 2,3,4,6-TETRACHLORO-
4 23760 BENZENE, 1,2,3,5-TETRACHLORO-4-ETHOXY-
5 30906 1,3,5-METHENO-1H-CYCLOPROPA[A]PENTALENE, 1,1A,4,5,5A,6-HEXACHLORODOC*
6 39763 HALOWAX 1001
7 39759 HALOWAX 1099
8 10334 ETHANE, 1,1,2,2-TETRACHLORO-
9 20547 PHENOXATHIIN, 10,10-DIOXIDE

Rank  Formula                              M Wt  B Pk   Purity   Fit   RFit
1     C6 H2 O CL4                           230   232      796   991    798
2     C6 H2 O CL4                           230   232      782   988    782
3     C6 H2 O CL4                           230   232      745   954    745
4     C8 H6 O CL4                           258   232      641   828    738
5     C10 H4 CL6                            336   230      348   644    501
6                                             0   230      294   577    479
7                                             0   230      267   498    499
8     C2 H2 CL4                             166    83      247   715    247
9     C12 H8 O3 S                           232   232      207   615    299

Rank     Ret Time     B P Int      US Par 1     US Par 2        C A S  #
1          ___          ___         0.5660        ___           4901-51-3
2          ___          ___         0.5870        ___            935-95-5
3          ___          ___         0.6990        ___             58-90-2
4          ___          ___         0.6970        ___          56818-02-1
5          ___          ___         0.6990        ___          69743-71-1
6          ___          ___          ___          ___          58718-67-5
7          ___          ___          ___          ___          39450-05-0
8          ___          ___         0.7200        ___             79-34-5
9          ___          ___         0.6790        ___            950-47-0
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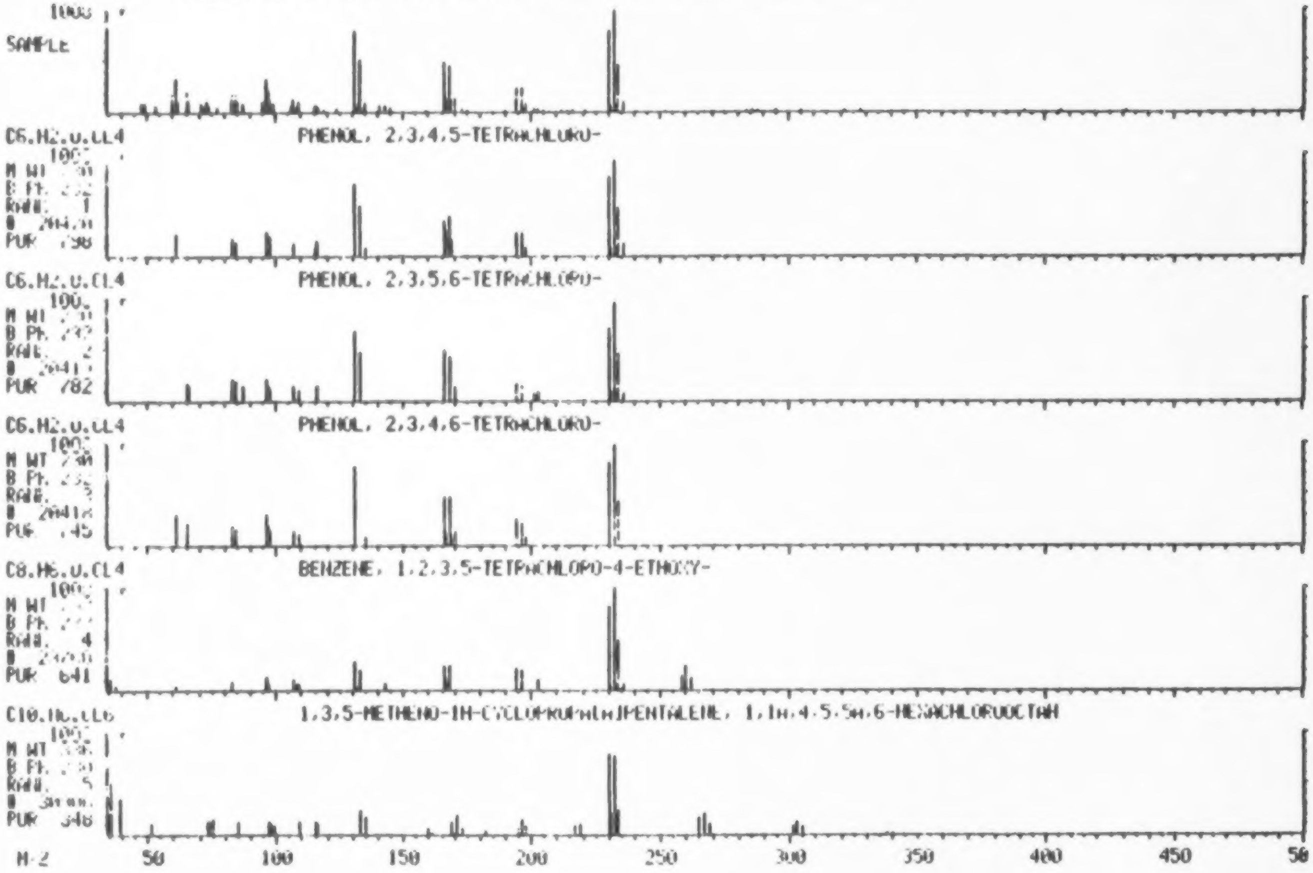
COMPOSITE MIBAS SAMPLE
PEAK 7
LIBRARY SEARCH REPORT

LIBRARY SEARCH　　DATA: 56?015 #1353　　BASE M/Z: 232
05/09/86 10:21:00 + 15:53　　CALI: 5G0010CAL32 # 4　　RIC: 253951.
SAMPLE: 41702-06-14 COMP WATER EXTRS #1-7 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #30779 HE/30/ I:300/ 60(4)-320@12(20)

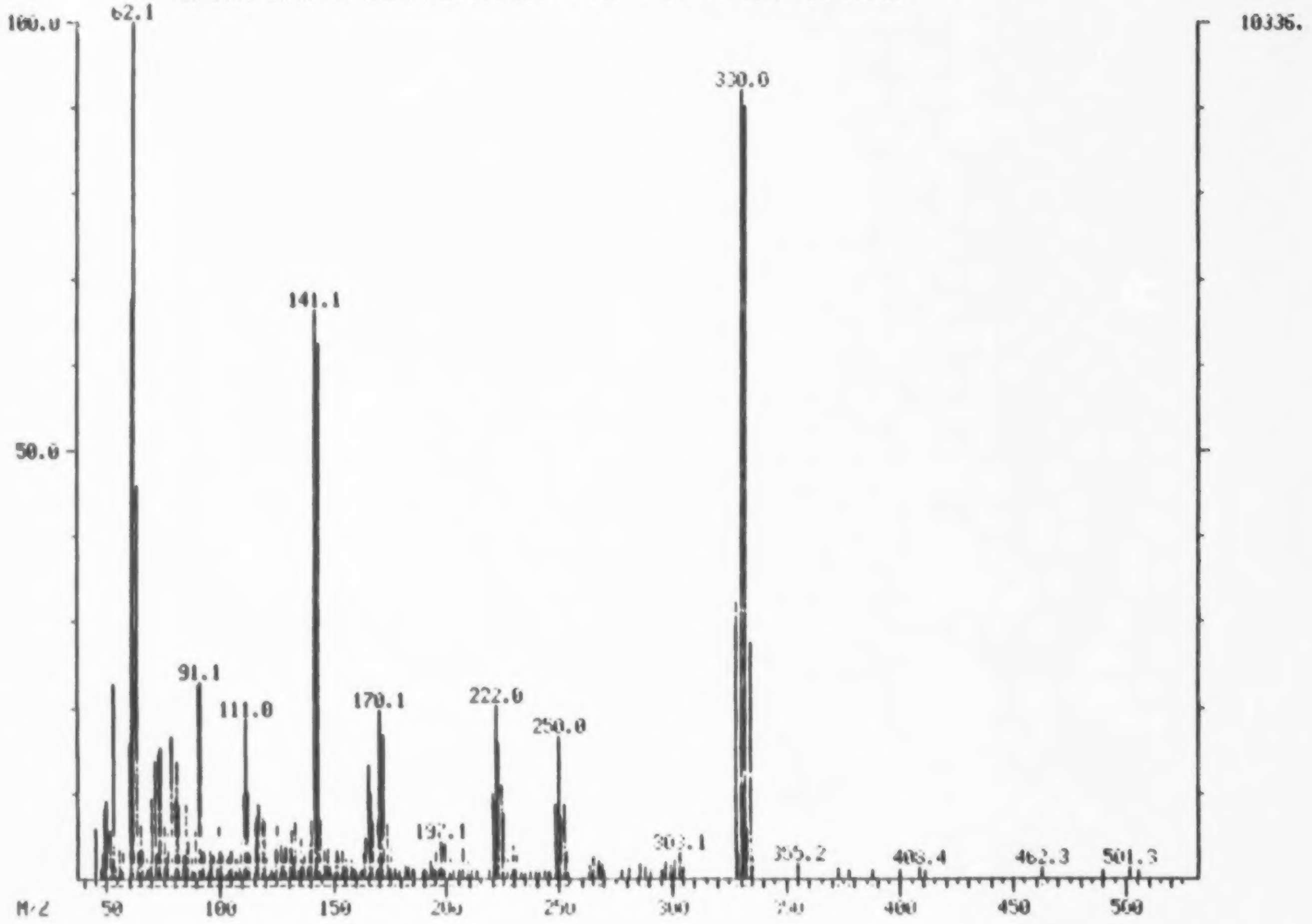


COMPOSITE MIBAS SAMPLE
PEAK 7
COMPARISON LIBRARY MASS SPECTRA

MASS SPECTRUM
05/09/86 10:21:00 + 16:51
SAMPLE: 41702-06-14 COMP WATER EXTRS #1-7 2.0UL SPLITLESS 10-2 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I:300/ 60(4)-320@12(20)

DATA: 560015 #1435
CALI: 56091060L32 #4

BASE M/Z: 62
RIC: 155332.



COMPOSITE MIBAS SAMPLE
PEAK 8
MASS SPECTRUM

```
Library Search                       Data: 568015 #1435         Base m/z:    62
05/09/86 10:21:00 + 16:51            Cali: 568010CAL22 #    4   RIC:     127743.
Sample: 41702-06-14 COMP WATER EXTRS #1-7 2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI,GC/MS 30MX 25MM DB5 #36779 HE(30) 1(300) 60(4)-320@12(20)

39763 spectra in LIBRARY NB searched for maximum PURITY
  260 matched at least 3 of the 16 largest peaks in the unknown

Rank In.        Name
1 30337 PHENOL, 2,4,6-TRIBROMO-
2 30662 DIACENAPHTHO[1,2-B:1',2'-D]THIOPHENE
3 32519 S-TRIAZINE, 2-(CHLOROMETHYL)-4,6-BIS(TRICHLOROMETHYL)-
4 30665 ANTHRACENE, 9,10-DIHYDRO-9,10-DIPHENYL-
5 30253 KETONE, 11-BROMOTRICYCLO[8.2.2.24,7]HEXADECA-4,6,10,12,13,15-HEXAEN*
6 39531 PHOSPHINE, TRIS(PENTACHLOROPHENYL)-
7 34698 ANTHRACENE, 9,10-DIHYDRO-9,9,10-TRIPHENYL-
8 30650 SPIRO[CYCLOHEXANE-1,3'-[3H]INDOLE]-2'-CARBOXY-O-TOLUIDIDE, 7'-METHY*
9 30520 9H-FLUORENE, 9-(DIPHENYLMETHYLENE)-

Rank   Formula                                      M.Wt  B.Pk   Purity   Fit   RFit
1      C6 H3 O BR3                                   328   330      707   987    707
2      C24 H12 S                                     332   332      307   560    345
3      C6 H2 N3 CL7                                  361   326      250   532    376
4      C26 H20                                       332   332      238   567    302
5      C16 H17 O BR                                  328   330      234   564    265
6      C18 CL15 P                                    772   328      220   506    357
7      C32 H24                                       408   330      215   549    280
8      C22 H24 O N2                                  332   332      212   654    272
9      C26 H16                                       330   330      206   452    293

Rank     Ret Time     B P Int      US Par 1     US Par 2        C.A.S. #
1          ____         ____        0.8410        ____          118-79-6
2          ____         ____        0.3020        ____          203-42-9
3          ____         ____        0.6430        ____        30361-97-8
4          ____         ____        0.4020        ____          603-58-7
5          ____         ____        0.1100        ____        18931-44-7
6          ____         ____        0.5240        ____        16716-14-6
7          ____         ____        0.3750        ____          809-40-5
8          ____         ____        0.0120        ____        18391-96-3
9          ____         ____        0.4930        ____         4709-68-6
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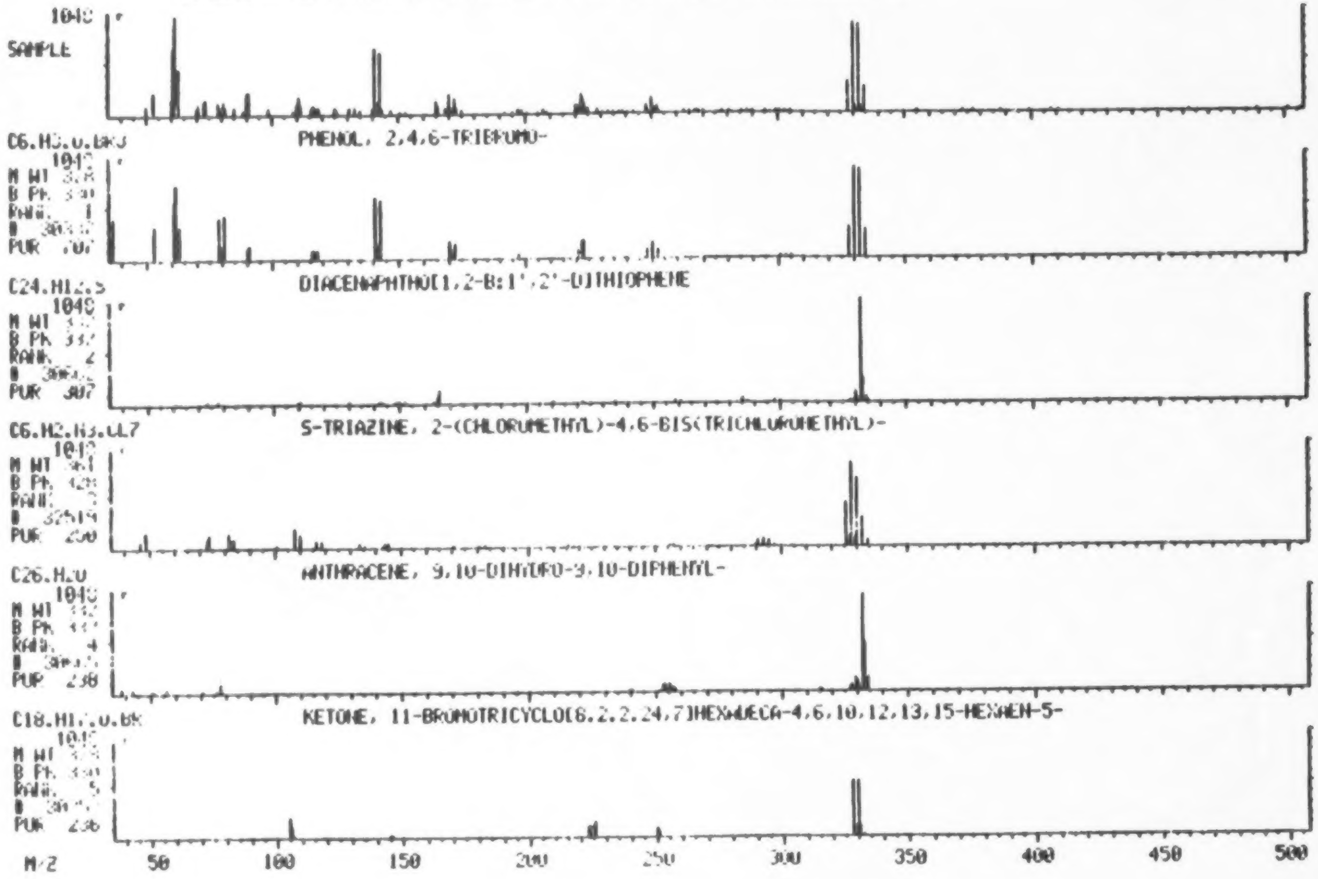
COMPOSITE MIRAS SAMPLE
PEAK 8
LIBRARY SEARCH REPORT

LIBRARY SEARCH
05/09/86 10:21:00 + 16:51
SAMPLE: 41702-06-14 COMP WATER EXTRS #1-7 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE(30) I(300) 60(4)-320@12(20)

DATA: 568015 #1435
CALI: 568010CAL32 # 4

BASE M/Z: 62
RIC: 127743.

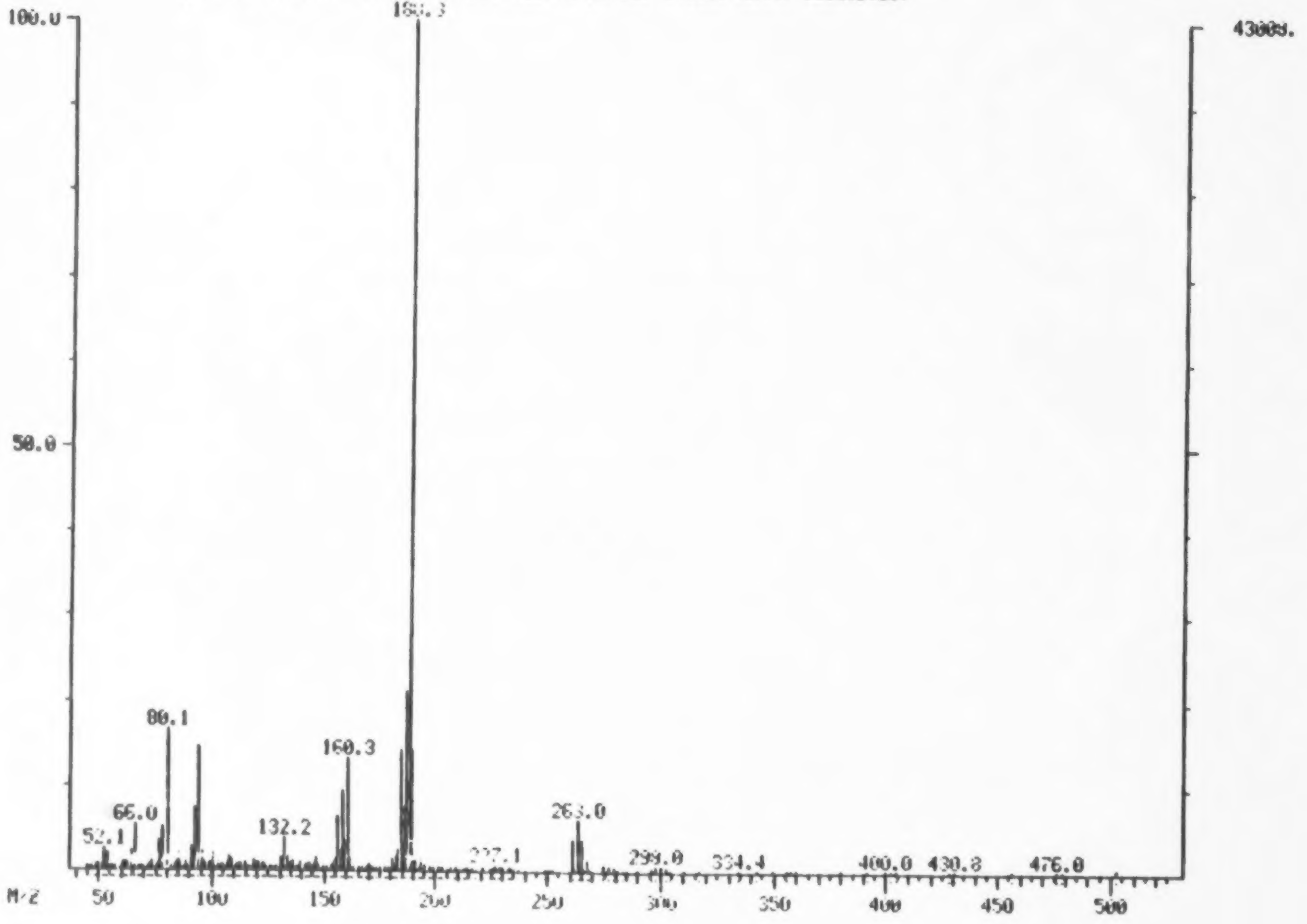


COMPOSITE MIBAS SAMPLE
PEAK 8
COMPARISON LIBRARY MASS SPECTRA

MASS SPECTRUM
05/09/96 10:21:00 + 10:21
SAMPLE: 41702-06-14 COMP WATER EXTRS #1-7 2.0UL SPLITLESS 10-7 @ 1100 EV
CONDS.: EI,GC/MS 30MX.25MM DB5 #33779 HE(30) I(300) 60(4)-320@12(20)

DATA: 5E9415 #1563
CALI: 5E0910CAL32 #4

BASE M/Z: 188
RIC: 175616.



COMPOSITE MIBAS SAMPLE
PEAK 9
MASS SPECTRUM

```
Library Search                Data: 568015 #1563        Base m/z:  188
05/09/86 10:21:00 + 18:21     Cali: 568010CAL32 #   4   PIC:     141567
Sample: 41702-06-14 COMP WATER EXTRS #1-7 2.0UL SPLITLESS 10-7 @ 1100 EV
Conds.: EI/GC/MS 30MX.25MM DB5 #36779 HE(30) 1(30) 60(4)-320@12(20)

35763 spectra in LIBRARYNB searched for maximum PURITY
  149 matched at least 4 of the 16 largest peaks in the unknown

Rank In.      Name
1 14326 PHENAZINE, DECAHYDRO-
2 14062 PYRROLO[2,3-B]INDOLE, 1,2,3,3A,8,8A-HEXAHYDRO-3A,8-DIMETHYL-, (3AS-*
3 14075 BENZENE, 1-(1-CYCLOHEXEN-1-YL)-4-METHOXY-
4 14122 SELENIDE, ETHYL 1-METHYL-1-PENTEN-3-YNYL
5 14021 4H-PYRIDO[1,2-A]PYRIMIDIN-4-ONE, 6-ETHYL-2-METHYL-
6 14055 DIBENZOTHIOPHENE, 1,2,3,4-TETRAHYDRO-
7 14036 3H-NAPHTH[illegible]THIOPHEN-2-ONE, 4,5-DIHYDRO-
8 14061 CYCLOHEXANONE, PHENYLHYDRAZONE
9 14018 3H-PYRAZOL-3-ONE, 2,4-DIHYDRO-4,4-DIMETHYL-2-PHENYL-

Rank  Formula                                 M Wt  B.Pk   Purity   Fit   RFit
1     C12 H18 N2                               190   188     500    774    604
2     C12 H16 N2                               188   188     472    806    537
3     C13 H16 O                                188   188     451    784    538
4     C8 H12 SE                                188   188     425    739    500
5     C11 H12 O N2                             188   188     424    732    504
6     C12 H12 S                                188   188     421    690    491
7     C11 H8 O S                               188   188     42     761    515
8     C12 H16 N2                               188   188     40     720    528
9     C11 H12 O N2                             188   188     384    816    451

Rank    Ret Time    B.P. Int     US Pat 1    US Pat 2        C.A.S. #
1        ____        ____         ____        ____         72361-10-5
2        ____        ____        0.6740       ____          4085-16-1
3        ____        ____        0.6630       ____         20758-60-5
4        ____        ____        0.4450       ____         25125-48-7
5        ____        ____        0.6340       ____         38022-28-2
6        ____        ____        0.6990       ____         16587-33-0
7        ____        ____        0.3590       ____         10245-79-1
8        ____        ____        0.7230       ____           946-82-7
9        ____        ____        0.4620       ____         17694-06-3
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COMPOSITE MIBAC SAMPLE
PEAK 9
LIBRARY SEARCH REPORT

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LIBRARY SEARCH                                   DATA: 560015 #1563          BASE M/Z: 188
05/09/86 10:21:00 + 18:21                        CALI: C050186C1 32 #   4    RIC:    141567.
SAMPLE: 41702-06-14 COMP WATER EXTRS #1-7 2.0ML SPLITLESS 1U /2 * 1100 EU
CONDS.: EI,GC/MS 30MX.25MM DB5 #38779 HE 30, 1-300) 60(4)-320@12(20)
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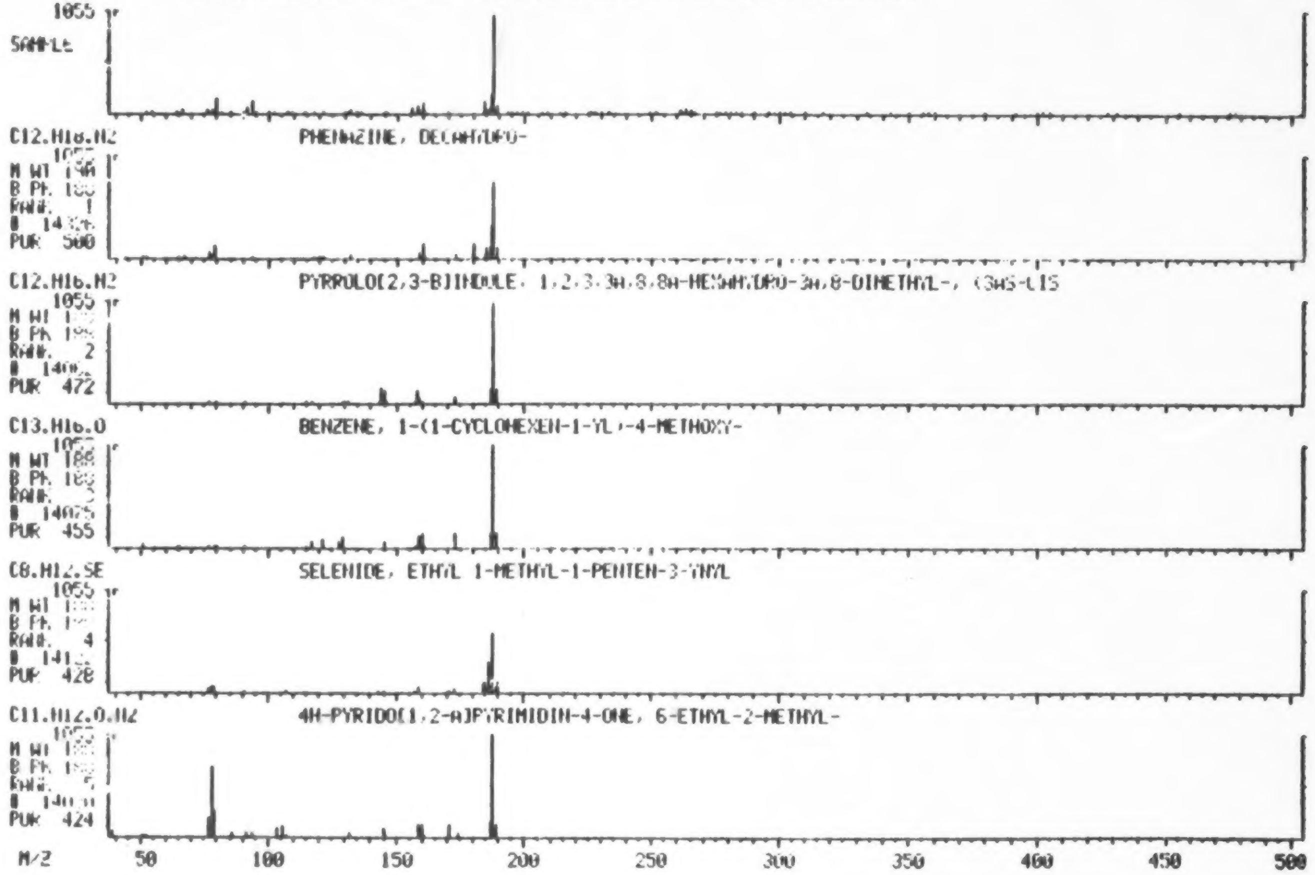
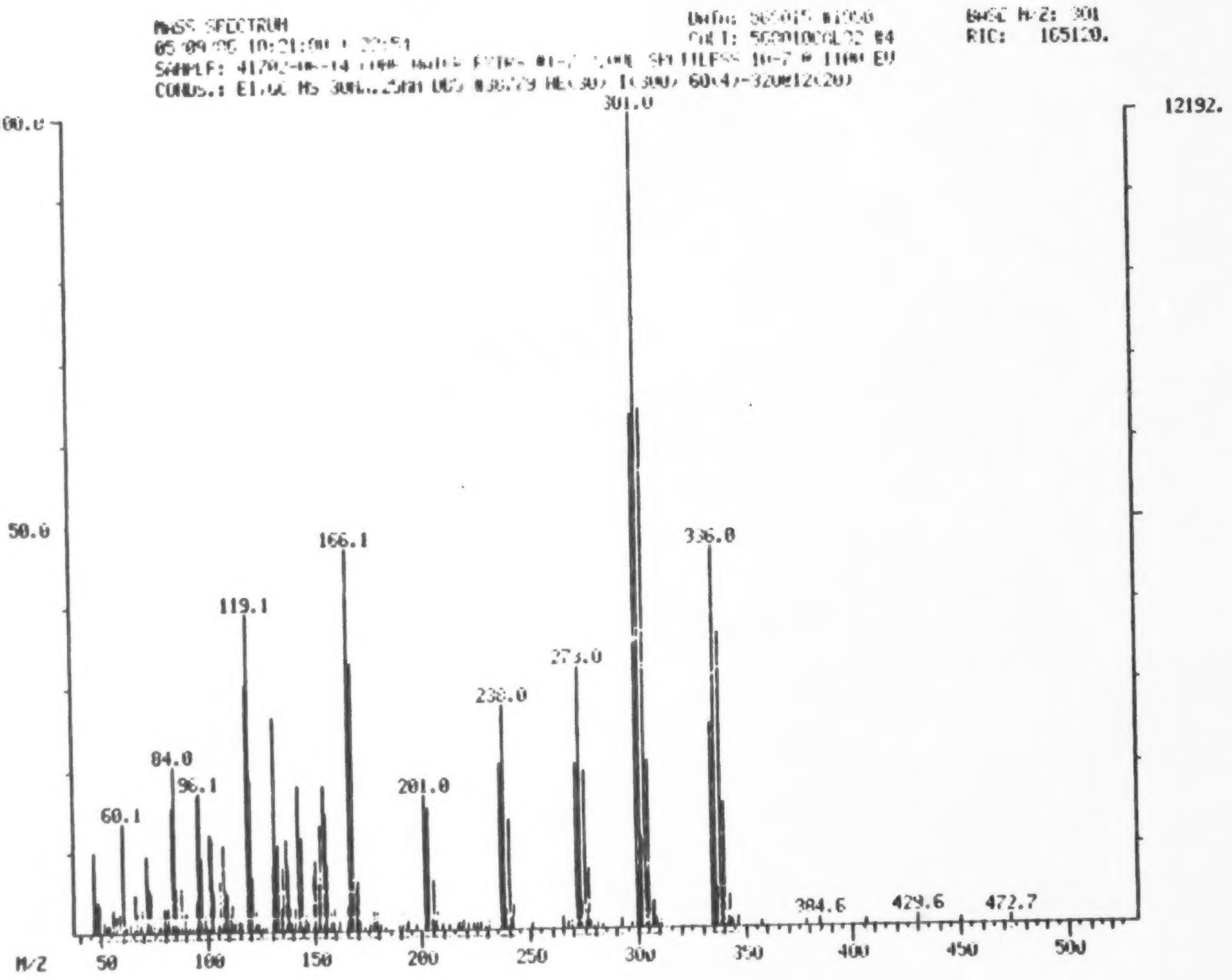


COMPOSITE MIBAS SAMPLE
PEAK 9
COMPARISON LIBRARY MASS SPECTRA



COMPOSITE MIRAS SAMPLE
PEAK 10
MASS SPECTRUM

```
Library Search               Data: 568015 #1950        Base m/z:  301
05/09/86 10:21:00 + 22:54    Cali: 568010CAL32 #   4   RIC:     144383.
Sample: 41702-06-14 COMP WATER EXTRS #1-7 2.0UL SPLITLESS 10-7 @ 1100 EV
Conds   ELMC MS 30MX.25MM DB5 #35779 HE(30) I(300) 60(4)-320@12(20.

39763 spectra in LIBRARY NBS searched for maximum PURITY
  119 matched at least 7 of the 16 largest peaks in the unknown

Rank In         Name
1 30378 1,4-BENZENEDICARBOXYLIC ACID, 2,3,5,6-TETRACHLORO-, DIMETHYL ESTER
2 32381 BENZOIC ACID, 2,3,5,6-TETRACHLORO-4-[(METHOXYMETHYLAMINO)CARBONYL]-*
3 31506 BENZOIC ACID, 2,3,5,6-TETRACHLORO-4-[(METHYLTHIO)CARBONYL]-, METHYL*
4 35640 FORMAMIDE, N,N'-[1,4-PIPERAZINEDIYLBIS(2,2,2-TRICHLOROETHYLIDENE)]B*
5 27807 3,1-BENZOXAZEPINE, 2-(P-BROMOPHENYL)-
6 30927 1,4-DITHIIN, 2,5-BIS(4-CHLOROPHENYL)-
7 27806 3,1-BENZOXAZEPINE, 7-BROMO-2-PHENYL-
8 31651 TUNGSTEN, TETRAKIS( ETA 3-2-PROPENYL)-
9 29750 STANNANE, (4-BROMOPHENYL)TRIMETHYL-

Rank   Formula                                 M Wt  B.Pk    Purity     Fit    RFit
1      C10 H6 O4 CL4                            330   301       334     703     459
2      C11 H9 O4 N CL4                          359   301       329     703     410
3      C10 H6 O3 S CL4                          346   301       303     715     387
4      C10 H14 O2 N4 CL6                        432   303       281     745     350
5      C15 H10 O N BR                           299   301       251     730     304
6      C16 H10 S2 CL2                           336   336       239     567     329
7      C15 H10 O N BR                           299   301       236     724     291
8      C12 H20 W                                348   303       219     550     349
9      C9 H13 BR SN                             320   305       214     506     351

Rank     Ret Time       B.P. Int      US Par 1     US Par 2       C A S  #
1          ____           ____        0 6990         ____        1861-32-1
2          ____           ____        0 7000         ____       14419-01-3
3          ____           ____        0 5200         ____        3765-57-5
4          ____           ____        0 7280         ____       26644-46-2
5          ____           ____        0 6030         ____       19062-91-0
6          ____           ____        0 4620         ____        2244-77-1
7          ____           ____        0 5970         ____       19062-90-9
8          ____           ____        0 7270         ____       11077-54-6
9          ____           ____        0 6510         ____         937-11-1
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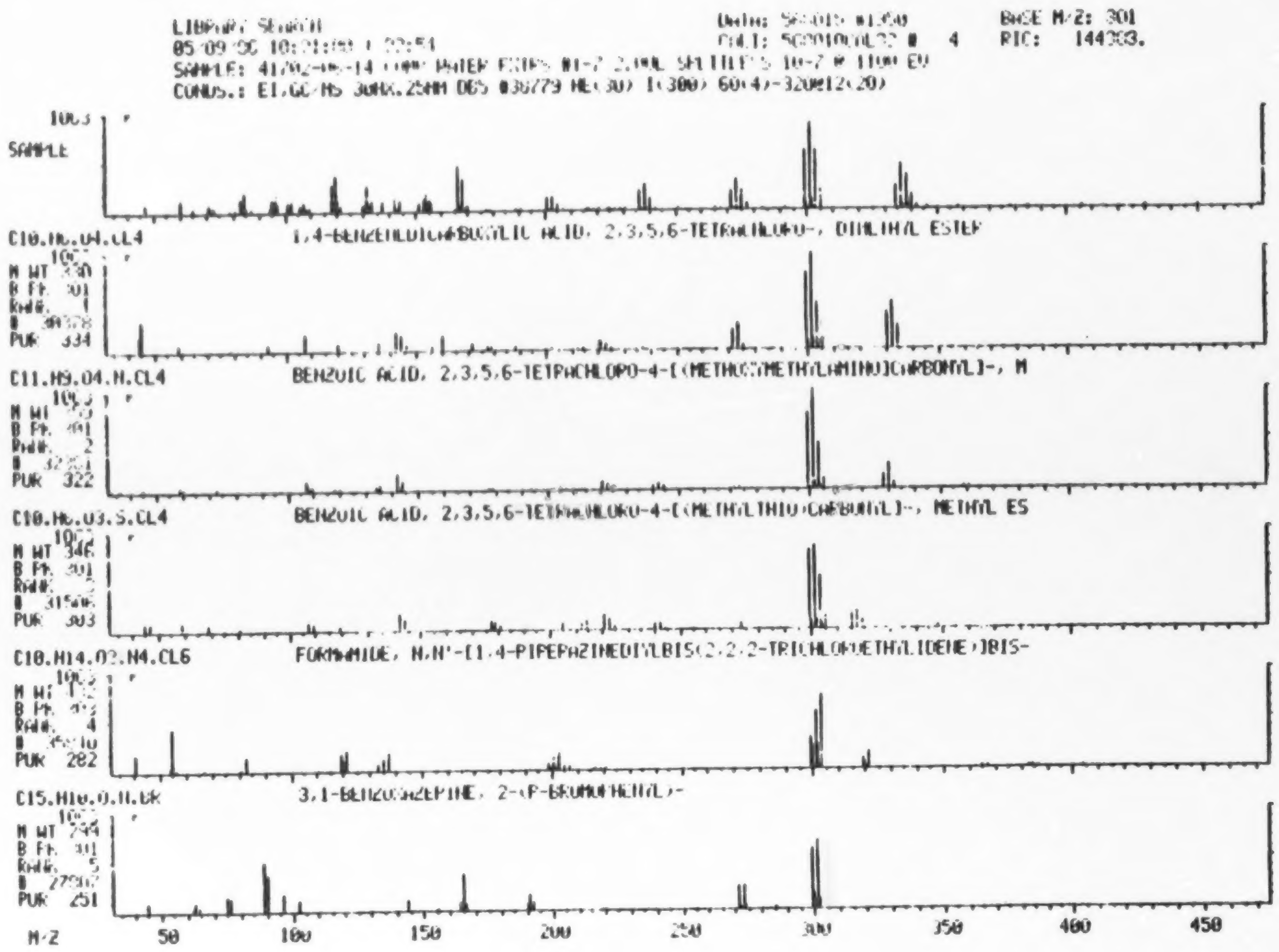
COMPOSITE MIRAS SAMPLE
PEAK 10
LIBRARY SEARCH REPORT



COMPOSITE MIBAS SAMPLE
PEAK 10
COMPARISON LIBRARY MASS SPECTRA